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The Plant Disease Reporter is issued as a service to plant pathologists throughout the United States. It contains reports, summaries, observations, and comments submitted voluntarily by qualified observers. These reports often are in the form of suggestions, queries, and opinions, frequently purely tentative, offered for consideration or discussion rather than as matters of established fact. In accepting and publishing this material the Crops Research Division serves merely as an informational clearing house. It does not assume responsibility for the subject matter.

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Paul R. Miller

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CONTENTS

EFFECT OF THE ADDITION OF ORGANIC AMENDMENTS TO SOIL ON ROOT KNOT OF TOMATOES I. PRELIMINARY REPORT

Leander F. Johnson[1]

Abstract

Mature, dried crop residues, chopped to about 1/8 inch particle size were incorporated into pots of soil infested with Meloidogyne sp. Rates of residue addition equivalent to 5 and 10 tons/acre-furrow-slice were used. At various time-intervals (incubation periods) after residue addition, tomato seedlings were transplanted into the infested soils. All 11 residues significantly reduced the number of nematode galls per plant; some residues were more effective than others. In general, more control of nematode infection occurred after 30 weeks than after shorter periods of incubation. A 95 percent reduction in number of galls/plant was obtained in lespedeza hay-treated soil after 30 weeks of incubation.

Adding organic amendments to soil for controlling plant diseases has received considerable attention. It was shown by Fellows (2) in 1929 that the addition to infested soil of each of several kinds of organic matter such as chicken manure, alfalfa stems and leaves, potato flour, and others, greatly reduced the severity of take-all disease of wheat. King et al. (3) in 1934 found that heavy manuring of cotton land either prevented the development of Texas root rot or delayed it until a crop was matured. A reduction in severity of many other plant diseases has been obtained by utilizing animal manures and crop residues. Recently, Tolmsoff and Young (7) obtained considerable reduction of Verticillium wilt of potatoes by adding barley or oat residues to infested soil.

Very little information is available with respect to the effect of organic amendments on plant diseases caused by nematodes. One striking example of reduction in nematode infection by the addition of organic amendments was reported in 1938 by Linford et al. (5). They found that additions of chopped green pineapple plants, coarse grass, and cane sugar reduced the number of galls on pineapples caused by the root-knot nematode. Additions of pineapple plants approximately equivalent to 50, 100, and 150 tons per acre-foot of soil gave progressively greater reductions in numbers of galls. They believed that decomposition of the amendments resulted in the build-up of nematode capturing fungi, non-trapping fungal parasites of nematodes, predacious nematodes, and predacious mites. Duddington (1) reported that infection of oats by the cereal root nematode was reduced when chopped cabbage leaves were incorporated into infested soil prior to planting. He also found that the addition of dung plus Dactylaria thaumasia, a nematode capturing fungus, was better for control of the potato root nematode than dung alone. Recently, Lear (4) obtained significant reductions in populations of three types of nematodes when castor bean pomace was added to infested soil.

In view of these results it is apparent that more investigations of organic-amendment effects on nematode populations are desirable. This is a preliminary report on the influence of different crop residue-amendments on the development and severity of root knot of tomatoes.

MATERIALS AND METHODS

Meloidogyne sp. originally found on tobacco roots was cultivated and maintained in a greenhouse bed of fine sandy loam soil. Mature, dried crop residues, chopped to about 1/8 inch particle size were mixed thoroughly with aliquots of this infested soil. Residues were added at the rates of 0.5 and 1.0 pounds/100 pounds of soil, roughly equivalent to 5 and 10 tons/acre-furrow-slice. The treated soil previously was uniformly fertilized with 6-12-12 fertilizer at the rate of 0.2 pounds/100 pounds of soil. Six-inch pots containing the residue-treated soils were incubated in the greenhouse or placed outside the greenhouse and exposed to natural environmental conditions. The soil was watered when necessary to maintain adequate moisture

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levels. After various periods of incubation, 5-week-old tomato seedlings (Rutger variety) were transplanted into the treated soils. Four pot replicates were used for each residue-time treatment. After 6 weeks of growth the tomato plants were washed free of soil and examined for root-knot nematode infection. Data taken were of three types: 1) green weight of whole plants, 2) actual number of nematode galls per plant, and 3) a root-knot infection index obtained by placing the plants in visual infection classes.

EXPERIMENTAL RESULTS

Pots containing nematode infested soil treated with residue-amendments at rates of 5 and 10 tons per acre-furrow-slice were buried up to the rims in the field outside the greenhouse, and exposed to natural environmental conditions. The pots were divided into three groups, and tomatoes were transplanted after 2 weeks, 7 weeks, and 12 weeks of incubation, respectively. Reductions in the severity of root knot occurred after all incubation periods (Table 1). Tomatoes transplanted into lespedeza hay-amended soils after 12 weeks of incubation had an infection index rating 60 percent lower than that of the infested control. When the indices of all three incubation periods were averaged it was found that all the residue-amendments significantly reduced root knot severity. There was some relationship between the degree of control and green plant weights; the highly significant reductions in disease severity in the 10 ton rates of many of the residue-amendments were reflected by significant increases in plant weights, especially after an incubation period of 2 weeks (Fig. 1).

FIGURE 1. Tomato plants from root-knot nematode infested soil containing crop residue-amendments. Upper left to right: sterilized control, lespedeza hay (10 tons), and lespedeza hay (5 tons). Lower left to right: wheat straw (10 tons), soybean hay (10 tons), and infested control.

The experiment was repeated in the greenhouse using other crop residues at the rate of addition equivalent to 10 tons per acre-furrow-slice. In this case incubation periods were 12, 20, and 30 weeks. Again, control of root knot was obtained with all residues tested (Table 2). In general, there was progressively more reduction in disease severity with longer incubation periods. Ninety-five percent reduction of root knot was obtained after 30 weeks of incubation with lespedeza hay amendment. It is believed that the differences in the number of galls/plant in the infested controls was caused by varying greenhouse temperatures. Tomatoes were grown in the greenhouse in winter, spring, and summer, corresponding to the 12, 20, and 30 week periods of incubation, respectively. Since greenhouse temperatures generally were lower in winter and spring, tomato plants grown during these periods were smaller, had fewer roots, and therefore had fewer galls.

Table 1. Severity of root knot of tomatoes (as measured by a root-knot index[a]) grown in nematode infested soil amended with various crop residues.

Residue amendment	Rate of addition (tons/acre)	Incubation period[b] (weeks) 2	7	12	Mean
Sterilized control	--	0.0	0.0	0.0	0.0
Infested control	--	4.0	3.5	3.3	3.6
Wheat straw	5	3.0	2.0	2.5	2.5
	10	2.3	2.8	2.8	2.6
Oat straw	5	2.5	3.0	2.8	2.8
	10	2.0	2.8	1.8	2.2
Soybean hay	5	2.5	2.5	2.5	2.5
	10	2.5	2.5	2.0	2.3
Lespedeza hay	5	2.8	2.5	1.8	2.4
	10	2.0	2.5	1.0	1.8
Fescue hay	5	3.0	3.0	1.3	2.4
	10	3.3	3.0	2.5	2.9
Stable bedding wheat straw	5	3.0	3.8	2.3	3.0
	10	3.0	2.5	2.8	2.8
L.S.D. 5%	--	0.7	0.6	1.1	0.5

[a]Root-knot index: 0 - no infection, 1 - slight, 2 - moderate, 3 - heavy, and 4 - very severe infection.

[b]Five-week-old tomato plants transplanted to pots of infested soil 2, 7, and 12 weeks after residue addition, respectively.

Table 2. Severity of root knot of tomatoes (as measured by actual numbers of nematode galls/plant) grown in nematode infested soil amended with 10 tons/acre of various crop residues.

Residue amendment	Incubation period[a] (weeks) 12	20	30	Mean
Sterilized control	0.0	0.0	0.0	0.0
Infested control	18.0	43.8	98.8	53.5
Corn	9.3	9.8	9.0	9.4
Oat straw	3.0	10.0	9.0	7.3
Lespedeza hay	5.5	10.5	4.8	6.9
Soybean hay	4.3	12.5	6.8	7.9
Tomato	6.8	6.8	7.3	7.0
Buckwheat hay	11.8	18.0	10.3	13.4
Cotton	11.8	16.3	13.8	14.0
Red Clover hay	5.5	7.8	10.0	7.8
L.S.D. 5%	6.2	11.4	23.7	13.9

[a]Five-week-old tomato plants transplanted to pots of infested soil 12, 20, and 30 weeks after residue addition, respectively.

Other experiments were conducted in the greenhouse several times using lespedeza hay and oat straw as amendments. There was an average reduction of 60 percent in root knot severity of tomatoes transplanted after 10 weeks of incubation.

DISCUSSION

It is possible that the addition of crop residues to infested soils for control of the root-knot nematode might have some practical application. However, since the results given here

are all based on pot experiments, extensive field trials are necessary before any recommendations can be made. The practical value certainly would be limited if it were found that high rates (10 tons/acre or more) of residue addition are necessary for adequate control.

An understanding of the mechanism of control might offer a better approach in the utilization of this method. Duddington (1) and Linford et al. (5) have shown a definite stimulating effect of organic matter on predacious fungi and predacious nematodes. It seems reasonable that an increase in numbers and activity of predacious organisms might be responsible for at least some of the reductions in nematode populations. However, other factors might also be involved, such as that reported by Patrick and Koch (6) who found toxic substances released during residue decomposition. A basic study of residue decomposition in relation to its effects on the soil microflora and microfauna would be highly desirable.

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CULTURAL AND PATHOGENIC VARIABILITY OF RHIZOCTONIA SOLANI ISOLATES FROM COTTON-GROWING AREAS OF NEW MEXICO[1]

Charles R. Maier[2]

Summary

Greenhouse tests of Rhizoctonia solani isolates from New Mexico cotton-growing areas revealed a number of strains of the fungus. In all, 245 isolates were separated into 10 strains on the basis of their cultural variation and on their ability to parasitize cotton. There was limited cultural variation among the 10 strains, but their pathogenicity ranged from non- to highly-pathogenic. These strains maintained their respective levels through several experiments, performing similarly on both Upland (Acala 1517C) and American-Egyptian (Pima 32) cotton.

INTRODUCTION

Many papers have been published regarding phases of parasitism of crop plants by Rhizoctonia solani Kuehn, but the work on the strains of the fungus affecting cotton has been largely neglected. Le Clerg (7) found wide differences in the ability of various R. solani isolates from sugar beets and potatoes to rot sugar beet slices. An even wider range of pathogenic variability occurred on cross-inoculation. Several isolates from older potato plants were non-pathogenic to sugar beets, but sugar beet isolates as a group were rather severe on potatoes. Sanford's results (8) were in general agreement with those of Le Clerg. He isolated several parasitic races from sclerotia and stem lesions on potatoes, and reported a wide variation in pathogenicity.

Sprague (9) differentiated five physiological races by inoculation to various crop hosts, including a number of susceptible grasses, while Cherewisk (4) reported strains parasitic to several broadleafed plants, but not pathogenic to any of the grasses. Boosalis (2) studied 16 races of the pathogen on five varieties of soybean, and concluded that the pathogenicity of R. solani was quite variable, and general rather than specific. Kommendahl and Young (6) reported a wide variation in the persistence of R. solani in the soil in the presence of various hosts. Blair (1) showed that R. solani isolates were suppressed by the addition of certain types of fresh organic matter, but not all to the same degree. Kernkamp et al. (5) found considerable differences in the ability of R. solani isolates to survive treatment with nine chemicals, and demonstrated measurable differences between isolates and their parent cultures in response to the chemical. These reports, and the investigations of Chen (3) involved the study of R. solani on a wide variety of hosts; their results are almost as varied as the fungus itself.

PROCEDURES AND TECHNIQUES

Each isolate of R. solani obtained from cotton-growing areas in New Mexico, from both diseased plants and soil samples, were transferred to three sets of Petri plates containing carrot agar, potato-dextrose agar, and nitrate-dextrose agar. After the cultures had covered the dish and sclerotia formed, the cultural characteristics of the fungus were recorded.

Pathogenic variability was determined by subjecting Acala 1517C and Pima 32 acid-delinted cotton seed to the various isolates. The procedure was conducted as follows: greenhouse soil, mixed 3:1:1 with sand and peat moss, was autoclaved for 4 hours at 22 psi (165°C). This soil was added to 3-inch clay pots, previously steam-sterilized, to three-fourths their capacity. Four pieces of carrot agar containing an isolate was placed on the soil, covered with additional soil, and watered. Three days were allowed for isolates to spread and grow in the soil, then cotton seed was planted at a depth of 1 1/2 to 2 inches, and the pots watered.

Each isolate was replicated three times in randomized block design, and the results of disease grade obtained after 2 to 3 weeks were subjected to analysis of variance.

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[1] Journal Series, New Mexico Agricultural Experiment Station, University Park, New Mexico.

The investigation reported in this paper was begun by Mr. R. E. Hunter, formerly Assistant in Biology with the Department of Biology, New Mexico Agricultural Experiment Station, University Park, and completed by the author.

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Table 1. Cultural variation by mycelial and sclerotial characteristics of 10 strains of Rhizoctonia solani on three different media.

Strain	Mycelial Characteristics[a]			Sclerotial Characteristics[a]		
	Carrot Agar	Pot. Dex. A.	Nit. Dex. A.	Carrot A.	Pot. Dex. A.	Nit. Dex. A.
1	mostly submerged	profuse, aerial and submerged	aerial and submerged	very few, aerial	none	submerged
2	mostly submerged	aerial and submerged	mostly aerial	few, submerged	none	aerial and submerged
3	submerged	aerial and submerged	aerial and submerged	very few, submerged	profuse, mostly aerial	few, submerged
4	submerged	mostly submerged	aerial and submerged	none	submerged	aerial and submerged
5	submerged	aerial and submerged	mostly submerged	none	none	none
6	submerged	mostly submerged	mostly submerged	none	few, submerged	mostly submerged
7	submerged	aerial and submerged	mostly submerged	none	none	aerial and submerged
8	submerged	mostly aerial	aerial and submerged	none	none	very few, aerial
9	submerged	aerial and submerged	mostly submerged	none	profuse, submerged	aerial and submerged
10	mostly submerged	aerial and submerged	aerial and submerged	none	none	mostly submerged

[a] Characteristics based on three Petri plates each, through six transfers.

Table 2. Percent loss of Upland and American-Egyptian cotton seedlings due to 25 selected isolates of Rhizoctonia solani.

Isolate	Disease loss based on Pct. seedlings[a]				Severity[b] class
	Upland Cotton		American-Egyptian Cotton		
	Trial 1	Trial 2	Trial 1	Trial 2	
1	100**	100**	91**	100**	HP
2	100**	98**	98**	99**	HP
3	100**	99**	98**	100**	HP
4	98**	7	40	78*	MP
5	100**	93**	90**	100**	HP
6	14	6	25	10	NP
7	15	5	30	10	NP
8	84**	75*	88**	97**	HP
9	99**	89**	89**	100**	HP
10	100**	100**	100**	85**	HP
11	33	2	44	12	NP
12	45	36	32	51	SP
13	41	28	38	56	SP
14	48	39	52	68*	SP
15	29	11	31	15	NP
16	67*	68*	70*	79*	MP
17	87**	68*	73*	78*	MP
18	97**	90**	97**	100**	HP
19	33	16	29	17	NP
20	34	10	47	42	SP
21	60*	33	46	37	SP
22	31	22	63*	54	SP
23	96**	61*	84**	100**	HP
24	44	17	53	80**	SP
25	72*	74*	72*	78*	MP
Check	25	16	31	14	-

[a]Mean of three replicates per trial.

[b]Rated highly, moderately, slightly, or non-pathogenic.

**Significant at 0.01 level.

*Significant at 0.05 level.

RESULTS AND CONCLUSIONS

Cultural Variation of R. solani:

Culture isolation of 1180 diseased cotton seedlings produced 245 cultures of R. solani. These isolates were grouped into 10 strains or races on the bases of their cultural variation and on their ability to parasitize cotton seedlings.

All strains appeared similar on carrot agar, growing rapidly with little to no production of sclerotia and mostly submerged vegetative mycelium. The same strains, when placed on potato-dextrose agar, rapidly produced both aerial and submerged mycelium; four of them produced sclerotia, and three of these produced submerged sclerotia. The sclerotia produced by the four strains differed in size and color. When plated on nitrate-dextrose agar, eight of the isolates produced submerged sclerotia, and seven of those also produced aerial sclerotia. As on potato-dextrose agar, the sclerotia produced by members of the various strains differed in size and color. These cultural differences are presented in Table 1.

Pathogenic Variability of R. solani:

Twenty-five selected isolates, representing the 10 strains, from cotton-field collections were tested in the greenhouse. They ranged from non-pathogenic to highly pathogenic (Table 2). Isolates obtained from different localities exhibited the same wide range of pathogenicity. Seventeen of the 25 isolates in the tests were quite stable, maintaining their pathogenicity through several transfers and through four tests. Three of the isolates, Nos. 23, 8, and 17, differed only slightly over the four tests in their degree of pathogenicity. The remaining five isolates, Nos. 4, 14, 22, 21, and 24, varied from non-pathogenic to pathogenic, and in some cases to highly pathogenic.

DISCUSSION

An increased knowledge of the variability of a pathogenic fungus with a wide host range could lead to the discovery of effective control measures. R. solani isolates exhibited such a great degree of variability, however, that finding effective control measures does not appear imminent. Preliminary experiments, which are as yet incomplete, indicate an extremely variable response of R. solani isolates to treatment with various chemicals. This would appear to bear out the findings of Kernkamp et al. (5) on isolates from other crop hosts. It appears that delayed planting, crop rotation, and other cultural practices offer the best means of keeping losses due to R. solani to a minimum.

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WILTING OF POINSETTIA, A DISEASE OF UNKNOWN ETIOLOGY

C. M. Tompkins

Summary

A disease of potted poinsettia plants, propagated from cuttings, is described. The chief symptoms are sudden wilting, yellowing, abscission of leaves and the colored bracts, and often premature death. Studies have failed to indicate the cause and control of the disease.

INTRODUCTION

Among the diseases affecting poinsettia (Euphorbia pulcherrima) potted plants grown under glass in the San Francisco Bay region of California, none is more devastating than one which causes sudden wilting of the foliage, followed frequently by premature death. During the past 10 years, this disease has increased in severity on all varieties, especially Indianapolis and Barbara Ecke Supreme, with losses of 20 to 60 percent of the crop. Studies on symptoms and etiology of the disease are discussed briefly.

SYMPTOMS OF THE DISEASE

The earliest symptoms of this disease are visible on one or several of the lowest leaves, consisting of sudden wilting, yellowing, and upward curling along the edges, followed by abscission (Figure 1). In turn, the next lower leaves become affected. Soon the remaining leaves and bracts become involved, leaving only a bare main stem surmounted by a cluster of true flowers (Figure 2). There is no visible external discoloration of the main stem, crown, or roots. Internally, however, the main stems of diseased poinsettia plants always show a slight vascular discoloration. Usually, but not always, affected plants die within 1 week after the first symptoms appear. Those plants which linger on are unsalable. Although the disease may be found on a few poinsettia plants in pots and pans early in the season, the principal damage occurs when the bracts commence to change color. Diseased poinsettia plants may be found in any part of a glasshouse; their distribution shows no particular pattern. Sometimes the disease does not appear on some poinsettia plants until late in December, after delivery has been made to retail florist shops.

STUDIES ON ETIOLOGY

In the autumn of four consecutive years, internal tissue fragments taken from recent, naturally-infected, potted poinsettia plants (varieties Barbara Ecke Supreme, Indianapolis, Ecke Pink, and Ecke White) were planted on poured plates of potato-dextrose, malt extract, and water agars. Colonies of but one fungus, Fusarium oxysporum, developed in a few days, but occasionally a few plates remained sterile. Approximately 125 diseased plants of the two red-flowering varieties and 25 each of the pink-and white-flowering varieties were examined culturally each year.

Six isolates of the fungus were tested in the glasshouse in two successive years on healthy red-and white-flowering seedling poinsettia plants and on two principal commercial red varieties, Barbara Ecke Supreme and Indianapolis, derived from cuttings. All plants were grown in 5-inch pots of steam-sterilized soil. Methods of inoculation were: 1) rooted cuttings were dipped in a spore suspension of the fungus before potting in soil; 2) roots of rooted cuttings were clipped before dipping in a spore suspension; and 3) a spore suspension was poured on the surface of the soil. The last method was the only one of the three mentioned that was used in inoculating seedling poinsettia plants. Each inoculated lot consisted of 25 plants, while 200 or more controls were provided. No infection occurred in any of the inoculated lots, but some control plants derived from cuttings developed the disease. All plants were held until they reached the flowering stage. These tests indicated that all isolates tested were non-pathogenic.

Further tests were conducted in the glasshouse to determine whether fungicidal treatment or special nutrition of cuttings might reduce or eliminate the disease. Unrooted, softwood cuttings of Barbara Ecke Supreme and Indianapolis, from presumably healthy stock plants, were used in lots of 50 in 1957. After dusting five lots with Cuttstart 1/2 X (a commercial rooting

FIGURE 1. Poinsettia potted plants grown from cuttings. At left, healthy plant; at right, naturally diseased plant showing wilting, yellowing, and upward curling of the lower leaves.

FIGURE 2. Naturally diseased poinsettia plants grown from cuttings, showing advanced symptoms of the disease, including leaf drop.

powder), the cuttings were placed individually in 2-inch pots of sterile sand. Similar lots received an instant dip in Chloromone (a commercial, liquid product for rooting) at 1/4, 1/2, 3/4 and full strength, and indolebutyric acid (1 to 3,000). In other lots, cuttings were completely immersed in clorox solution (5 percent) and aerated for 20 minutes, or in boric acid solution (1 part B per million parts of water), and then placed in sand. Some of the latter two lots received additional treatment with Cuttstart 1/2 X, Chloromone, or indolebutyric acid. Approximately 500 cuttings were placed in sterile sand, without treatment, as controls. After rooting, all cuttings were placed singly in 5-inch pots of sterile soil of acid reaction and were watered moderately as required. During the ensuing 3 1/2 months, the disease appeared in se-

vere form in all treated and control lots.

Apparently healthy poinsettia plants in 5-inch pots, in lots of 20, received weekly applications either of boric acid solution (1 part B per million parts of water), Hoagland's complete nutrient solution, Es-Min-El (a commercial product which contains no N, P, or K, but does contain Mn, Cu, Zn, Fe, and B), at the rate of 1 teaspoonful per gallon of water, Nu-Iron (4 teaspoonfuls per gallon of water), and Nu-Manese (10 grams per gallon of water), applied as a soil drench. Plants of additional lots were sprayed with Nu-Zinc (9 grams per gallon of water). Control plants of both varieties were watered only as required. The disease appeared in substantial form in all treated and control lots, and no beneficial effects from the fertilizer application were apparent.

During the autumn of 1957, expressed juice from the leaves, stems, and roots of affected poinsettia plants selected at random from both treated and control lots was wiped on the leaves of apparently healthy Barbara Ecke Supreme, Indianapolis, and seedling poinsettia plants, with carborundum[1] as an abrasive. Also inoculated were young seedling plants of bean (Phaseolus vulgaris), cabbage (Brassica oleracea var. capitata), carrot (Daucus carota var. sativa), celery (Apium graveolens var. dulce), China aster (Callistephus chinensis), cucumber (Cucumis sativus), Datura stramonium, globe amaranth (Gomphrena globosa), lettuce (Lactuca sativa), bell pepper (Capsicum frutescens var. grossum), spinach (Spinacia oleracea), sugar beet (Beta vulgaris), Turkish tobacco (Nicotiana tabacum), and N. glutinosa. All tests were negative.

DISCUSSION

Failure has attended all efforts thus far to reproduce this disease experimentally in the glasshouse by inoculation and to prevent its occurrence by the application of complete or incomplete nutrient solutions. Additional studies on the effects of environment, irrigation, potting soil mixtures, fertilizers used commercially and nutrition in general, and stock plants from which cuttings are taken, would seem to be desirable, if not imperative, because of the importance of the poinsettia crop to the flower industry. Until the cause and control of this disease are finally determined, it may well continue to be the major factor in determining success or failure of the poinsettia crop.

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[1] Rawlins, T. E., and C. M. Tompkins. 1936. Studies on the effect of carborundum as an abrasive in plant virus inoculations. Phytopathology 26: 578-587.

PRELIMINARY OBSERVATIONS ON THE INTERNAL CORK VIRUS COMPLEX IN THE SWEETPOTATO

E. M. Hildebrand[1]

Summary

In 1956 symptomed sweetpotato foliage was indexed for the presence of internal cork virus. By use of Scarlett O'Hara morning glory as the indicator plant and of the modified "squeeze-rub" rapid-transmission technique, virus isolates or strains were demonstrated in numerous sweetpotato varieties and seedlings collected in Maryland (at Beltsville) and in Mississippi, Louisiana, Texas, Oklahoma, Kansas, and Missouri. On the basis of incubation period and symptoms produced on morning glory there appeared to be three isolates or strains: strain #1, with an incubation period of 5 to 8 days and characterized by dwarfing, rugosity and vein-clearing followed by masking; strain #2, with an incubation period of 8 to 12 days and characterized by moderate to severe vein-banding followed by masking; and strain #3, with an incubation period of 10 to 15 days or even more, and producing mild vein-banding, netting and masking.

In 1955 Scarlett O'Hara morning glory was found to be ideal for indexing to show leaf-spot and root-spot lesions caused by the sweetpotato internal cork virus complex (1). Likewise, the entity which causes foliage symptoms of the internal cork virus complex on morning glory as well as on sweetpotato was demonstrated to be transmitted by aphids (6, 7). The possibility of a virus complex was suggested by the observation that this morning glory constantly developed several different symptoms. Furthermore, the possibility of mixtures of virus strains or of viruses was suggested by the overlapping of symptom responses which obscured the response differences on both the sweetpotato host and the indexing host.

Two syndromes have been distinguished in the sweetpotato virus complex, that is, internal cork and feathery mottle (4). Each syndrome in turn may perhaps be brought about by several virus strains or components. The sweetpotato viruses or strains involved are probably labile and hence unsuitable for in vitro study (2). This fact may account for the difficulty encountered in establishing their true identity.

This report gives the results of two experiments in which virus from symptomed sweetpotato hosts was transmitted to the morning glory indicator plant in an attempt to relate morning glory foliage symptoms to virus presence and virus identity in the sweetpotato. In a preliminary survey of sweetpotato viruses, symptomed sweetpotato foliage was collected at different locations in the States from Maryland to Georgia during August 1955. In dampened newspapers in a closed metal container many of the leaf blades dried out or discolored by the time of our return to headquarters 8 days later. However, the petioles were still turgid and readily indexed for virus on morning glory (3) and proved satisfactory for demonstrating virus presence.

Two sweetpotato virus studies of the internal cork syndrome were conducted on symptomed foliage of selected seedlings and varieties indexed on Scarlett O'Hara morning glory: 1) Foliage Indexing Experiment I used leaf specimens collected from the sweetpotato breeding plots on East Farm at Beltsville, and 2) Foliage Indexing Experiment II used leaf samples from sweetpotato plots visited in Mississippi, Louisiana, Texas, Oklahoma, Kansas, and Missouri.

FOLIAGE INDEXING EXPERIMENT I

Several types of chlorotic spots characterized the foliage symptoms on sweetpotatoes at Beltsville: chlorotic spot (CS), ringspot (RS), concentric ringspot (CRS), feather (F), oakleaf (OL), and chlorotic spot bordered by purple (CS_p). In this experiment two morning glory plants were used for isolation of virus from each distinctive symptom pattern on each variety or seed-

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ling tested. The samples of symptomed foliage were collected from 36 different seedlings or varieties, stored overnight in a moist chamber, and indexed the following day on morning glory by means of the modified "squeeze-rub" rapid transmission technique.

The indexing results on morning glory were recorded three times at weekly intervals. Three virus isolates or strains are indicated based on differences in symptoms and length of incubation period: strain #1, with an incubation period of 5 to 8 days and characterized at first by severe dwarfing, rugosity, and vein-clearing, followed by almost complete masking; strain #2, with an incubation period of 8 to 12 days characterized by moderate to severe vein-banding followed by masking; and strain #3, with an incubation period ranging from 10 to 15 days or more and characterized by a mild vein-banding and/or netting and masking. At the time of this study only three isolates or strains of internal cork virus were recorded.

At 7 days, 27 of the 36 clones showed strain #1 of the virus. At 2 weeks, 16 or 44 percent of the clones showed symptoms of strain #2 for the first time, all but three of which had shown symptoms of strain #1 the week before. A mild vein-banding or netting symptom, designated as strain #3, appeared during the second week or later in six of the otherwise symptomless indicator plants.

FOLIAGE INDEXING EXPERIMENT II

Foliage Indexing Experiment II was conducted about 2 weeks after the Beltsville experiment on materials collected from sweetpotato plots in Mississippi, Louisiana, Texas, Oklahoma, Kansas, and Missouri. The symptomed foliage material collected on this trip was essentially the same as that indexed at Beltsville in Experiment I. Leaf blade deterioration of some of this material occurred in mail transit and these leaves were unusable for the indexing tests. However, the petioles remained turgid and were, as in Experiment I, satisfactory for isolation purposes.

All the indexing tests were performed on 1 day. As before, the observations were made at approximately weekly intervals after indexing. From the six States 60 specimens representing 37 distinct clones were indexed. Of these, 41 specimens indexed positive for strain #1 and 28 for strain #2, with 16 overlapping to leave 6 specimens negative and two indexed for strain #3.

DISCUSSION

From this study it appears that three or more strains may be involved, based on severity of symptoms and length of incubation period. It appears that precocious morning glory strain #1 is frequently in mixture with strain #2, the latter appearing in a high frequency of cases (13/27 in Experiment I; 17/41 in Experiment II). However, it can be noted that strain #2 may occur in the absence of strain #1, as happened 3 times in Experiment I and 11 times in Experiment II, and, vice versa, strain #1 may occur in the absence of strain #2, as happened 14 times in Experiment I and 24 times in Experiment II.

Subsequent studies indicate that many virus strains may be involved or are in mixture in the internal cork syndrome. These studies indicate that one component of strain #1 is the virus most commonly associated with and isolated from the internal cork root lesions on sweetpotato. Strain #2 appears to be the virus component most commonly associated with sweetpotato foliage symptoms in the absence of the internal cork root lesions (5). More work is yet required to demonstrate virus strains by Scarlett O'Hara morning glory. To date none of the strains have been established as pure.

It has been demonstrated (2) that cysteine, a reducing chemical, when applied to the root lesion material of plants with the internal cork syndrome made mechanical transmission possible by protecting the virus from chemical change. When in vitro, the use of cysteine was impractical because of the low in vitro longevity and a very low dilution end point of the virus strains.

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NEMATODES ASSOCIATED WITH CROWN-BLIGHT DISEASED CANTALOUPS IN DESERT SOILS[1]

Robert B. Marlatt[2], Donald J. Morton[3] and Robert T. McKittrick[2]

Abstract

Soil and roots were collected from under cantaloup vines with crown blight and from others which appeared healthy. Cantaloups were planted in part of the soil in the greenhouse and nematodes were collected from the remainder and from the roots. Later, nematodes were extracted from the greenhouse soil.

There was no apparent relationship of a field history of crown blight to occurrence of the disease in greenhouse plants grown in soil from disease areas.

Nematodes obtained from soil and root samples included species of Acrobeles, Aphelenchus, Cephalobus, Dorylaimus, Eucephalobus, Pratylenchus, Prismatolaimus, Rhabditis, Trichodorus and Tylenchorhynchus. No relationship could be found between nematode genera which might be parasitic and crown blight. More nematodes of all genera were obtained from soils under healthy vines than from diseased vines.

Extracting nematodes from root samples resulted statistically in less variability than use of soil samples and gave generally the same results.

INTRODUCTION

This investigation was begun as an attempt to find the cause of premature dying of cantaloups in Arizona's desert production areas. Beginning at the bases of the runners, leaves die progressively outward toward the runner tips. Since the base of the main stem is referred to as the crown, the disease is called crown blight. The disease has previously been described in 1954 (4) and later (1, 2, 3, 5); its cause is unknown. The main objects of these experiments were to determine whether crown blight is related to the presence of plant parasitic nematodes and whether the disease is soil-borne. In conjunction with this, the predominant non-parasitic nematodes associated with cantaloups were also noted.

MATERIALS AND METHODS

Collection of Soil Samples:

Soil and roots were collected from mature vines of the PMR45 variety in the Yuma Valley. The surface inch or two of soil was scraped from around a plant and about 10 pounds of soil and roots were removed to a depth of approximately 9 inches. The sample was immediately placed in a numbered plastic bag and then into a cool icebox for transport to Mesa. In each of six fields samples were collected from six dying plants within a crown-blighted area and six normal plants from an adjacent healthy area. Each sample was obtained from a different bed at a distance of 10 or 20 feet from the others. From five other fields one each of healthy and diseased vines were sampled, which completed the collection of 82 samples.

Each sample was put through a quarter-inch screen and then thoroughly mixed by hand. One hundred grams of soil were saved for a Baermann funnel and the remainder stored in the plastic bag at 55° F until used in the greenhouse experiment.

First Recovery of Nematodes:

Nematodes were collected daily for 3 days from each Baermann, refrigerated at 40° F for

[1]Arizona Agricultural Experiment Station Technical Paper No. 546. The authors are indebted to A. L. Taylor, H. W. Reynolds and J. H. O'Bannon for some of the nematode identifications.

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a few days to several weeks, and then identified at the United States Department of Agriculture nematology laboratory, Tempe, Arizona. Specimens were examined for plant-parasitic forms only; no counts were made.

Greenhouse Experiment:

Cantaloups were grown in each soil sample to determine whether crown blight is soil-borne and as a means of retaining, or perhaps increasing, the nematode populations. Each screened, mixed sample was placed in a sterile, 8-inch pot and planted with surface disinfected PMR45 cantaloup seeds. The 82 pots were randomized twice during the experiment. Plants were thinned to two per pot, periodically tied to stakes and kept growing vigorously by adding nutrient solutions. Greenhouse air temperatures ranged from 50° to 99° and daily modes ranged from 69° to 79° F.

One of the two plants in each pot was selected at random and examined at six intervals for symptoms and severity of disease. Records were kept of the death of cotyledons, types of leaf injury, the total number of leaves on the main runner (larger than a 25-cent-piece) and the number of leaves one-half or more dead. From the latter two figures a mortality percentage was calculated and used as an estimate of crown-blight severity. Percentages were compared by analysis of variance.

Second Recovery of Nematodes:

Following approximately 6 months of continuous cantaloup culture, the potted soil samples were prepared for a second series of Baermann funnels. All live roots were screened from the soils, macerated in a Waring Blendor for 30 seconds with 60 ml of water and processed in separate Baermann funnels. Soils were thoroughly mixed, 100-gram samples were obtained with a Jones sample splitter and placed in Baermann funnels. At least 10 ml were drawn from each funnel five times over a 2-week period, with the first sample being drawn after 72 hours. The liquid from each funnel was stored at 40° F while waiting to be concentrated.

Concentrating Nematode Suspensions:

At the end of the 2-week period, 75 to 100 ml of liquid containing nematodes had been drawn from each funnel. It was necessary to remove all but 4 ml of water from the samples so that they could be preserved in small vials and mailed for identification. For a quantitative study, excellent results were obtained by removing excess water with a pipette while observing the operation with a stereoscopic microscope. Since this process was slow for concentrating 164 samples, the following apparatus was used:

A fritted-glass filter was suspended in a sample which nearly filled a 100-ml beaker. The filter consisted of a glass tube 8 mm in diameter, flared to 30 mm at the end which contained a fritted-glass wafer. The filter was connected by a short piece of rubber pressure tubing to a glass stopcock with a 2-mm bore. This in turn was connected to a T-shaped glass tube.

Each unit thus consisted of the sample, filter, stopcock and T-tube. Nine of these units were joined with rubber tubing and connected, through a 1-liter filtering flask, to an aspirator filter pump (Fig. 1). Passing a small stream of water through the pump provided a vacuum adequate for the nine units. An average laboratory faucet could probably cause a vacuum sufficient for at least twice as many filtering units.

The distance between the fritted wafer and the bottom of the beaker determined the amount of liquid that remained after filtering.

After the nematodes had settled in a beaker, the vacuum was applied. Filters usually remained clean and fast because most of the residue remained on the bottom of the beaker, not on the fritted glass. After all but 4 ml had been drawn from the samples, occasionally a few nematodes remained attached to the filter. This problem was solved by attaching a rubber squeeze bulb to the end of the train of filters. With all stopcocks closed, the vacuum was broken by removing the stopper from the filtering flask and the rubber tubing was pinched between the flask and the train. Then with one stopcock open, the bulb was firmly squeezed, a few drops of water were forced back through the disc and the nematodes fell back into the 4-ml sample. This was repeated for each sample, the entire filtering process taking only 10 to 15 minutes. Using a pipette and microscope had required two or three times as long for just one sample.

Nematode Identification and Counting:

The 4 ml in each vial were poured into a flatbottomed dish marked by appropriate divisions. As many nematodes were removed from the dish as would fit under a cover glass on a

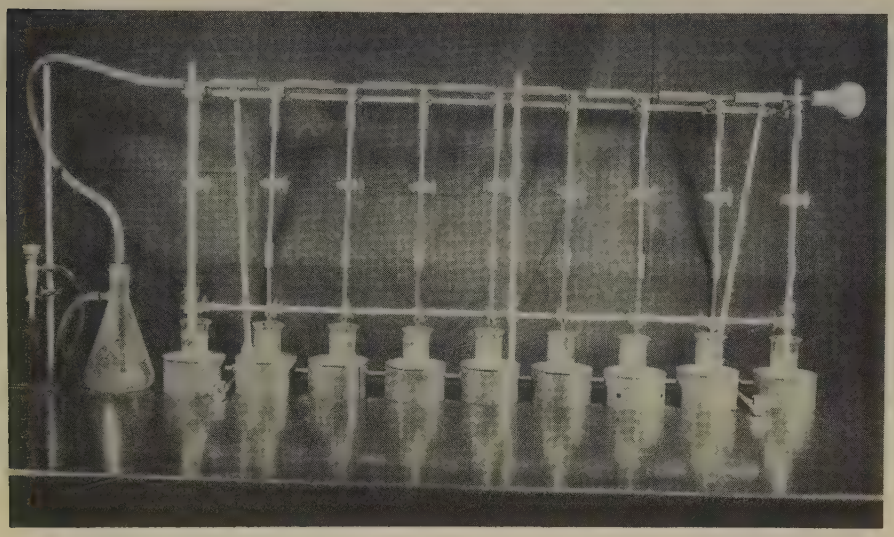

FIGURE 1. Apparatus for concentrating several water suspensions of nematodes simultaneously.

microscope slide. The area of the dish from which these nematodes were taken was used to determine the fraction of the total vial sampled. A smaller portion of a populous sample was needed to fill the space under a cover slip. The number of each nematode genus found under the cover glass was multiplied by the denominator of the fraction of the total vial used to estimate the population. This method was used so that one man could estimate the numbers of eight nematode genera in 168 samples within the time allotted for the experiment.

Analysis:

Only those fields from which 12 samples were collected were statistically compared. The estimated totals were transformed to square roots for a sounder analysis.

Analysis was first made of estimated numbers of nematodes in root and soil samples from healthy and diseased areas in each field, then data were combined for all fields. Degrees of freedom were as follows: among fields -- 5, crown blight versus healthy -- 1, crown blight versus healthy x fields -- 5, samples in fields -- 60. "Samples in fields" was used to test interaction and main effects.

RESULTS

Greenhouse Experiment:

Cotyledons had begun to die 37 days after planting. After 47 days more than three-quarters of the plants showed dead cotyledons and all were dead by 74 days after planting. There were more vines with dead cotyledons growing in soil from healthy areas than on plants in soil from crown-blight areas. It is therefore apparent that this cotyledon dying was not directly related to crown blight in the fields.

Many of the vines eventually developed the "yellow" type of crown-blight symptoms. An entire leaf lamina may gradually turn yellow, usually beginning at the tip. The blade eventually becomes necrotic and brown on the tip or side, with the brown area advancing toward the center and base. The petiole remains green until much of the leaf shows necrosis. Several plants exhibited "gray-green" crown-blight symptoms and others a kind referred to as the "brown" type.

Vines with gray-green killing first have basal leaves with gray-green necrotic margins which advance toward the center and base of the lamina. Dead margins are often preceded by small, necrotic spots at the hydathodes. There is no yellow halo between dead and living tissue and necrotic margins advance faster than with other types of crown blight, which may account for the gray-green color. Petioles remain turgid and green until the laminae are almost completely dead. Usually there is no unilateral chlorosis or necrosis of the petiole. Gray-green symptoms often appear before the brown type on the same plant.

Brown-type symptoms also begin at the leaf-blade margins but necrotic tissues are brown.

The brown, necrotic area advances slowly toward the center and base of the lamina, often preceded by a yellow halo. The necrotic areas are often wedge-shaped and petioles show symptoms soon after a brown wedge occurs. The side of a petiole under a brown wedge becomes chlorotic and often necrotic and brown. The brown type of crown blight is the most common and usually follows the others.

At present, all of the above symptoms are considered to be part of the crown-blight syndrome. The various symptoms occurred in approximately the same proportions on plants in "healthy" and in "crown-blight" soil.

The percentages of leaves one-half or more dead were analysed from readings made after 74, 105 and 123 days following planting. Analyses showed that there were no significant differences in percentages of dead leaves between vines growing in soils from crown-blight areas and those in "healthy" soil for any one field on any one date. When combining all fields for each reading date or all dates, again crown blight was not significantly greater in vines growing in soils from disease areas.

There was no apparent relationship of a previous field history of crown blight to leaf injury in the greenhouse. This verified failures during previous years to demonstrate soil transmission of the disease or to control it by soil fumigation in several greenhouse and field experiments.

Nematode Identification and Counting:

During the first recovery of nematodes, from field soil, occasional specimens of Pratylenchus, Trichodorus and Tylenchorhynchus species were found. Since actual counts were not made and the predominant non-parasitic forms were not reported, no analysis could be made.

In the second recovery of nematodes, the eight most commonly occurring genera and their estimated total numbers in the 164 samples were as follows: Acrobeles -- 95,340, Rhabditis -- 35,590, Cephalobus -- 17,891, Aphelenchus -- 13,709, Dorylaimus -- 12,761, Prismatolaimus -- 4,797, Tylenchorhynchus -- 4,127 and Eucephalobus -- 3,864. Only three of the genera, Acrobeles, Aphelenchus and Rhabditis, occurred with sufficient consistency for individual analysis; the other five were included for analysis of all genera.

Acrobeles

Root Samples: Significantly greater numbers of Acrobeles were found in healthy than in crown-blighted areas in four of the fields sampled. There was no interaction of field with healthy versus crown blight; hence, the field chosen did not affect the healthy versus crown-blight relationship. One field had significantly fewer Acrobeles than any of the other fields.

Soil Samples: The results in soil were generally the same as for root samples but the variability seemed to be higher in soil, even though soil samples were weighed and roots were not.

Aphelenchus

Root and Soil Samples: There were no significant differences found in numbers of Aphelenchus between healthy and crown-blighted areas nor between populations from field to field.

Rhabditis

Root Samples: No significant differences were found in Rhabditis numbers between healthy and crown-blight areas. There was significant variation in total numbers from field to field, but the similarities between healthy and crown-blighted areas were consistent.

Soil Samples: Although there were no differences evident within any individual field, the combined data showed significantly more of this genus in healthy than in blighted locations.

All Eight Genera Combined

Root and Soil Samples: The combined results are dominated by Acrobeles because of its relatively high total population. There were significantly more nematodes (most of them obviously non-parasitic) in healthy than in crown-blighted areas, with this difference being consistent from field to field. Two fields had significantly more total nematodes than the others. Variance for the soil samples was much greater than for the root samples.

Summarizing: There was no apparent relationship of the two possibly parasitic genera

(Tylenchorhynchus and Aphelenchus) to crown-blighted or healthy locations in the fields.

Since root samples generally gave the same results as soil samples, it might not have been necessary to collect nematodes from both. Root samples showed less variability than soil and might therefore be preferred if the nematodes under consideration would as likely be found on roots. This was unexpected in view of the fact that soil samples were weighed to 100 grams each and root samples were of various weights and sizes.

There were more nematodes in soil originally obtained from under healthy cantaloups than in soil from under crown-blighted vines. Possibly this was due to a more extensive root system on healthy plants, with conditions more favorable for an initially high nematode population. Also, perhaps soil from "crown-blighted" locations had characteristics which were less favorable for nematode development in the field, in the greenhouse, or in both places.

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AERIAL SURVEY FOR DUTCH ELM DISEASE

Abraham H. Epstein

The Wisconsin State Department of Agriculture has found aerial survey to be effective in scouting for the Dutch elm disease. Preliminary flights made over areas in which the disease was known to be established convinced the writer that this method would be ideal for detecting diseased trees in new areas on farms and in heavily wooded areas where it is difficult to find such trees using conventional transport and observation methods.

It was found that trees showing symptoms of the disease are quite conspicuous to an airborne observer for distances of up to 1 mile from altitudes of 500 to 1000 feet. Above 1000 feet, it became increasingly difficult to distinguish between elms and other tree species and, in addition, it was found that the observable area becomes much too large for the observer to cover accurately.

The survey was conducted during the first week of July in the southwestern counties, Green, Lafayette and Grant, and along the east bank of the Mississippi River from the Illinois line up to LaCrosse. This was a joint project between the State Department of Agriculture's Plant Industry Division, which furnished two observers, and the State Department of Conservation's Forest Management Division, which furnished a Cessna 180, the pilot and one observer.

The flight followed a predetermined course which was plotted on county highway maps on a scale of 2 miles per inch. Five parallel flights were made over the southern portions of Green, Lafayette and Grant counties. The first flight was made parallel to and 1 mile north of the Illinois-Wisconsin State line and the subsequent flight lines were laid out at 2-mile intervals north of this.

Each of the three observers was supplied with a complete set of the aforementioned maps for the two-fold purpose of keeping track of the position and for indicating the locations of suspect trees with a fair degree of accuracy. None of the locations, so indicated, were more than 1000 feet from the actual position of the tree on the ground. A total of 47 suspects were located in this area, along the Mississippi River and the LaCrosse area. Total flying time was approximately 6 hours and about 800 air miles were flown. The total area surveyed was roughly 1500 square miles.

The suspect locations were subsequently checked on the ground with the following results:

A. Dutch elm disease -- 2 locations in Green and Lafayette counties. The one in Lafayette County is some 30 miles west of any previously reported locations.
B. Verticillium sp. -- 5 locations.
C. Dothiorella sp. -- 7 locations.
D. Chemical injury -- 2 locations.
E. Girdled trees -- 23 locations. The majority of these were in farm stockyards, especially hog pastures.
F. Trees of other species -- 5 locations where trees were oak (oak wilt), and 3 locations were wild cherry which has somewhat the same foliage texture as elm.

In the opinion of the writer, the Cessna 180 is somewhat fast (135 miles per hour) to be optimum for such work. A subsequent flight in a Piper PA-12 (85 miles per hour) with only one observer proved to be more effective and permitted a more thorough scanning of the terrain but, of course, the distance covered per unit of time was less.

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EFFECTS OF TEMPERATURE AND CROP ROTATION ON THE OCCURRENCE OF BROWN SPOT OF CELERY IN SOUTHERN NEW YORK[1]

G. D. Lewis[2]

Summary

Brown spot of celery, caused by Cephalosporium apii Smith & Ramsey, is serious in southern New York only in summers having unusually high average temperatures. Lesions caused by this pathogen develop more rapidly at high temperature (75° F) than at low temperature (55° F). Control of this disease by crop rotation indicates that the pathogen is soil- or refuse-borne.

INTRODUCTION

Brown spot of celery, caused by Cephalosporium apii Smith & Ramsey (3), occurs sporadically on the mucklands of southern New York. Newhall (1) reports that Segall showed that differences in varietal susceptibility are considerable, thus partially explaining the irregular occurrence of this disease.

Segall (2) also suggested a relationship between high summer temperatures and an epiphytotic of brown spot in southern New York. He noted that in 1949, when the disease was severe, the departures of average temperatures from the long-term means for June, July, and August were +4.0, +4.6, and +4.2° F, respectively. In 1950, when there was little disease, the departures of average temperatures from the long-term means for June, July, and August were -1.2, -1.7, and -0.2° F, respectively. The optimum temperature for growth of C. apii has been shown to be 75° F (3). Segall suggested that average temperatures near the optimum for growth of the fungus are required for epiphytotics of the disease.

CORRELATION OF HIGH SUMMER TEMPERATURES WITH THE OCCURRENCE OF BROWN SPOT

The author observed the severity of brown spot in southern New York from 1953 to 1958. In addition, growers whose celery has been affected by this disease were questioned concerning the severity of the disease in 1951 and 1952. The year 1955 was the only one from 1951 to 1958 in which the disease was epiphytotic. An examination of weather data for these years (as recorded at Walden, New York) showed that the summer of 1955 was the only one in which the average monthly temperatures for July and August were noticeably above the long-term means for this region. Table 1 shows the departures of the average temperatures from the long-term means for the months of June, July, and August from 1951 to 1958.

LABORATORY STUDIES ON THE EFFECT OF TEMPERATURE ON INFECTION

The celery variety Cornell 619 was found to be highly susceptible to C. apii and was selected for use in laboratory tests. Preliminary experiments indicated that plants of this variety would develop numerous lesions after being sprayed with a conidial suspension of C. apii and placed in an incubation chamber for 8 hours at 70° or 80° F. Sixteen plants were sprayed with a conidial suspension containing spores from five isolates known to be pathogenic. The plants were placed in an incubation chamber at 80° F for 44 hours to insure infection. When the plants were removed from the incubation chamber, they were divided into groups of four. Three of the four groups were placed in illuminated constant-temperature chambers at 55°, 65°, and 75° respectively. The fourth group was placed on a greenhouse bench. The development of lesions was noted daily.

Table 2 shows the effect of temperature on the development of lesions. Lesions appeared the earliest and were numerous on the plants held at 75°. Lesions appeared the latest and were few in number on the plants held at 55°. By the time lesions began to appear on the plants held at 55°, most of the leaves of the plants held at 75° were dead, and the lesions on the plants held at 65° were larger than those on either the plants held at 55° or the plants placed on the greenhouse bench.

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Table 1. Departures of the average temperatures[a] from the long-term means[b] for the months of June, July, and August from 1951 to 1958.

Year	Departures from long-term means		
	June	July	August
1951	-2.0	-1.8	-1.7
1952	+1.1	+1.8	-0.8
1953	-0.2	-0.9	-0.8
1954	+0.1	-2.3	-3.1
1955	-2.6	+4.4	+4.1
1956	+0.1	-3.4	-2.2
1957	+2.6	-1.9	-4.0
1958	-6.1	-2.2	-1.1

[a] Recorded at Walden, New York.
[b] Long-term mean temperatures (°F): June, 67.1; July, 72.2; August 69.7.

Table 2. Effect of temperature on the development of lesions.

Temperature (°F)	Number of days from inoculation[a] to the appearance of lesions	Relative number of lesions
75	6	Numerous
65	8	Fairly numerous
55	11	Few
Greenhouse bench	10	Few

[a] Plants inoculated with conidial suspension and held at 80° F for 44 hours.

EFFECT OF CROP ROTATION ON SEVERITY OF BROWN SPOT

During the epiphytotic year of 1955 the writer observed that although one grower was experiencing losses of about 40 percent, there was less than 1 percent loss in the adjacent and nearby fields of another grower. This was particularly remarkable because both growers had planted seedlings of the same variety that had come from the same lot of seed and the same seedbed. Both growers were spraying with zineb, using similar equipment, and getting commercial control of early and late blight. However, the grower who experienced heavy losses grows celery in the same field every year, whereas the other grower rotates celery with onions, lettuce, and potatoes. The latter grower has never had an epiphytotic of brown spot, although his celery varieties are known to be susceptible. These observations are considered to be evidence that C. apii is soil- or refuse-borne but does not persist for long periods in the absence of celery.

DISCUSSION

The observations made by Segall (2) and the writer during the past 10 years indicate that brown spot of celery is serious in southern New York only during summers when the average temperatures are unusually high. Although infections may occur during cooler summers, it appears that high temperatures are required for lesions to enlarge rapidly. Occasional small lesions are of little economic importance, but the extensive lesions that develop under high temperatures can cause serious losses.

Growers have had little success in controlling this disease with fungicides. It is suggested that crop rotation be added to the use of resistant varieties for the control of brown spot.

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LEAF CURL--A TRANSMISSIBLE VIRUS DISEASE OF CITRUS

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Summary

Symptoms associated with a leaf curl disease of citrus are described. The causal agent was successfully transmitted by budding, and symptoms of leaf curl were produced on Caipira and Hamlin sweet oranges and on Eureka lemon, sour orange, shaddock, and grapefruit. Leaf curl is caused by a virus that appears to be a distinct entity. The causal virus may be more closely related to the psorosis virus group than to the other recognized viruses of citrus.

The Pera orange trees in a 20-year-old rootstock planting at the Limeira Citrus Experiment Station in São Paulo, Brazil were all severely pruned in 1953 as a means of rejuvenating the planting. Practically all trees responded to this treatment with almost double their previous production. There was one Pera orange tree on sweet orange rootstock, however, that appeared to be detrimentally affected following the pruning treatment. Its prior yield record of 1000 to 1500 fruits per year had made it one of the best trees in the rootstock planting. In the 2 years following pruning, the yield of this one tree dropped to 276 and then to 115 fruits.

The difference in the response of this tree in comparison with the adjacent tree in the planting is shown in Figure 1. The die-back of branches and sprouting give the diseased tree

FIGURE 1. Pera orange trees on sweet orange rootstock. A -- showing symptoms of leaf curl disease; B -- apparently healthy tree.

FIGURE 2. Caipira sweet orange seedling 2 months after inoculation with a bud from the leaf curl infected tree shown in Figure 1A.

gross symptoms similar to those described in Florida for "blight" (3). On close examination of the Pera orange tree it was found that the leaves were curled in a manner similar to those on young sprouts following heavy infestations of aphids. However, large aphid populations had not been observed on this tree and, if they had been, one would not have expected the aphids to attack every branch or to be so abundant on this tree and completely lacking on the adjoining trees.

In a series of observations it was found that the diseased tree produced abundant flowers that developed only a few fruits of small size. The sprouts on the branches were weak and

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broke off readily under slight pressure. Gum was found in the wood vessels, especially near the union of the sprouts and the old branches. Upon removal of bark from the main branches and trunk, the wood appeared to be channeled with some depressions suggestive of pitting.

TRANSMISSION TESTS

Buds from the diseased Pera orange tree were propagated as tops on nucellar seedlings of the following citrus varieties: 10 Caipira sweet orange, 5 Cleopatra mandarin, 5 Rangpur lime, and 5 Poncirus trifoliata. Growth from the Pera orange buds developed poorly and all manifested leaf curl regardless of the rootstock employed. Sprouts were allowed to develop on all the rootstocks. Leaves on the sprouts from the Caipira sweet orange rootstock all showed a light green color and often a yellow margin, as well as leaf curl. All the sprouts from the Cleopatra mandarin, Rangpur lime and Trifoliate orange rootstocks showed normal healthy growth with no leaf curl in a 10-month period.

Similarly, buds from the same diseased Pera orange were budded onto uncut seedlings of the same varieties mentioned above. The 10 seedlings of Caipira sweet orange in a 2-month period began to show yellowing of the old leaves followed by leaf drop and subsequent development of lateral sprouts. These sprouts then manifested typical leaf curl symptoms as shown in Figure 2. All five seedlings of each of the other citrus varieties employed developed apparently healthy growth, in spite of the fact that the bud used for inoculation remained alive.

The control plants for these tests consisted of a similar number of seedlings of each variety and similar treatments. The bud source used was the apparently healthy Pera orange tree adjacent to the diseased tree in the rootstock planting and the same tree as shown in Figure 1B. All plants of all varieties budded from this source developed apparently healthy growth.

The results of these initial tests demonstrated the transmissibility of the causal agent of leaf curl from Pera sweet orange to Caipira sweet orange nucellar seedlings.

In a second transmission test the Caipira sweet orange seedlings infected in the initial tests and showing the characteristic leaf curl symptoms were used as a source of bud inoculum. The results of the second test are summarized in Table 1.

The results given in Table 1 show that the causal agent of leaf curl was transmitted to several varieties of citrus, and the development of symptoms on Eureka lemon was not influenced by the rootstocks employed.

OTHER OBSERVATIONS

No detailed survey has been made to determine the occurrence and distribution of trees with similar symptoms of leaf curl in the many citrus plantings in the State of São Paulo. In conjunction with field trips, however, a 12-year-old Hamlin orange tree on Caipira sweet orange rootstock having leaf curl symptoms was found in Fazenda Reserva, Araras. Buds from this Hamlin orange were used to inoculate Caipira sweet orange and Rangpur lime seedlings. The leaf curl symptoms were produced on the Caipira sweet seedlings but not on the Rangpur lime seedlings. The transmissibility of the causal agent to sweet orange and the lack of symptom expression on inoculated Rangpur limes indicate the similarity of the causal agents in the leaf-curled Pera and Hamlin orange tree sources.

DISCUSSION

That leaf curl is transmissible and is caused by a virus is evident from the results presented. The relation of the virus causing leaf curl to other known citrus viruses is not yet entirely clear. Some gross aspects of infected trees suggest "blight" symptoms, but "blight" has never been successfully transmitted by budding and grafting (3). Tristeza, xyloporosis (cachexia), and exocortis viruses in sweet oranges on sweet orange rootstock do not cause the leaf curl symptoms observed. In addition, the diseased Pera orange tree is one of many trees in a rootstock planting that originated from a single tree source of budwood. In this planting none of the Pera orange trees on sweet lime or on Trifoliate orange rootstocks show any symptoms of xyloporosis or exocortis. Also, none of the trees in this Pera orange rootstock planting show any leaf or bark symptoms associated with strains of psorosis.

Leaf curl differs from the Satsuma dwarf disease of Japan in that the Satsuma dwarf leaf symptoms did not develop on sweet orange scions even when grafted onto Satsuma trees showing typical dwarf symptoms (6), whereas leaf curl as herein reported has been readily transmitted to sweet orange seedlings.

Table 1. Results of transmission of citrus leaf curl from Caipira sweet orange to various citrus varieties.

Age of Tops Yrs.	Mo.	Variety and Rootstock[a]	No. infected[b] / No. inoculated
1	6	Eureka lemon/Cleopatra	2/2
	3	Eureka lemon/Caipira sweet orange	2/5
	3	Hamlin orange/Caipira sweet orange	2/5
2		Caipira sweet orange/Caipira sweet orange	10/10
	3	Willow leaf mandarin/Caipira sweet orange	2/5
	3	Citron/Caipira sweet orange	2/5
	3	Marsh grapefruit/Caipira sweet orange	3/5
	3	Shaddock/Caipira sweet orange	2/5
3	6	Rangpur lime seedlings	0/10

[a] The tops as well as the rootstocks were of nucellar seedling origin.

[b] Infection based on presence of leaf curl symptoms in a 3-5 month period following inoculation.

No typical psorosis leaf or bark symptoms have been observed on the Pera orange source tree or on any of the test plants of the varieties inoculated. This would tend to eliminate the heretofore described psorosis virus strains from consideration. However, the psorosis virus group loosely includes many variations of symptom expression. Some of the symptoms associated with psorosis crinkly leaf and with infectious variegation (1, 2) may approach, but are not reported to reach, the severe degree of leaf curl found on the Pera and Hamlin orange trees reported in this paper. Infectious variegation and crinkly leaf have been associated with sour orange and lemon (4). Leaf curl also affects these hosts. However, trees with crinkly leaf in the lemon tops were noted (5) as causing bark symptoms typical of psorosis A on their sweet orange rootstock. It was suggested that crinkly leaf disease may result from a mixture of two viruses, one of which is psorosis A virus and the other a yet unidentified virus, or that crinkly leaf may even be a strain of psorosis A.

The exact identity of the leaf curl virus has yet to be determined. It appears to be a distinct entity that may be more closely related to the citrus psorosis virus group than to the other recognized viruses of citrus.

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HEAD SMUT OF SUDAN GRASS AND SORGHUM IN CALIFORNIA[1, 2]

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Summary

Head smut, caused by Sphacelotheca reiliana (Kuehn) Clint., is a disease of economic importance in Sudan grass (Sorghum sudanense) and in grain sorghum (S. vulgare) grown in California. The disease is widespread in Greenleaf Sudan grass commonly grown here as a certified seed crop. The symptomatology of the disease in Sudan grass is discussed and illustrated. Cross-inoculation tests with soil-borne spores from smutted Sudan grass and smutted grain sorghum, respectively, resulted in cross-infection of Sudan grass, sorgo and grain sorghum varieties. Corn was not infected by inocula from Sorghum species. An examination of the pedigrees of six Sudan grass varieties revealed the presence of the smut-susceptible Leoti sorgo in the parentage of five of them; two of which were highly susceptible and three were resistant or immune.

Since a report (10) in 1920 of head smut occurring in California, the disease has been known to recur only sporadically on corn, sorghum, and Sudan grass crops in this State. In past years head smut has been found occasionally in the Sacramento Valley, but, in general, losses attributed to the disease were considered insignificant. In recent years however, with the introduction of Greenleaf Sudan grass as a widely-grown seed crop, and the advent of hybrid grain sorghums, the prevalence of head smut has increased markedly. Both these commercially cultivated crops are very susceptible to Sphacelotheca reiliana under local growing conditions which seem to favor the natural perpetuation of the disease on volunteer hosts. During the past 3 years the disease has been seen frequently in fields of Sudan grass, grain sorghum, and occasionally corn grown in Butte, Colusa, Sacramento, San Joaquin, Solano, Sutter, and Yolo counties. On hybrid grain sorghums field infection levels of 15, 27, and 41 percent, as determined by plant counts, were observed. In Sudan grass, the disease was found to be co-extensive with the cultivation of this seed and hay crop.

HEAD SMUT OF SUDAN GRASS

Head smut (S. reiliana) on Sudan grass has been reported from the States of California, Texas, and Washington (18), and also from Russia (3). No account of experimental studies with the disease on Sudan grass has been found in the literature. This dearth of information implies that head smut has not been recognized as troublesome on this host. Evidence of this is found in a recent USDA bulletin on Sudan grass (4) which does not mention head smut as a disease of this crop. Another bulletin on sorghum diseases (9) merely states that Sudan grass is moderately susceptible to S. reiliana. In California, however, the disease is considered to be of economic importance. The losses are aggravated by stringent regulations which specify that even trace infections must disqualify seed crops from eligibility for seed certification.

Head smut on Sudan grass has occurred sporadically for a number of years on the variety Sudan 23 grown in California. In 1955 the new variety Greenleaf Sudan was introduced from Kansas to be grown as a certified seed crop. As the acreage of Greenleaf Sudan grass increased in central California, the disease gradually became widespread. In 1957-59 head smut was commonly found wherever this variety was grown. This increase in prevalence of head smut in Sudan grass probably is a result of the introduction of a susceptible variety into an environment which is favorable for the natural perpetuation of the disease. Consequently, head smut is now widespread on Greenleaf Sudan grass in several counties where large acreages are grown as a seed crop.

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[1] Contribution from the Agricultural Experiment Station, University of California, Departments of Plant Pathology and Agronomy, Davis, California.

[2] Acknowledgment is due to C. M. Volkman & Company of Woodland, California for supplying seed of Sudan grass and hybrid sorghum varieties.

The symptomatology of head smut in Sudan grass, in general, resembles that produced in grain sorghum. Usually, infection is not evident until the panicle emerges. At the heading stage smutted panicles emerge as compact, elongated sori, each covered by a white peridium (Fig. 1B). An entire panicle of Sudan grass may thus be displaced by a compact, curved, cylindrical sorus measuring several centimeters in length. Other infected panicles may be only partially displaced by sori (Figs. 1C, 3B), and in such instances sterility is usually part of the disease syndrome. Soon after emergence the fragile membrane is readily ruptured exposing dark spore masses and the shredded vascular tissues of the inflorescence (Fig. 2). Sporulation of the head smut fungus is not limited to the panicles however. Heavily infected plants often bear shredded culms (Fig. 3A), and tattered foliage showing fungus sporulation (Fig. 3C). Sporulation in the foliage has been observed in both Leoti sorgo and in Greenleaf Sudan grass. Such symptoms are evidence of the systemic nature of fungus ramification in susceptible host tissue.

FIGURE 1. Head smut (Sphacelotheca reiliana) of Greenleaf Sudan grass. A -- healthy inflorescence; B -- compact, elongated smut sorus which emerges in place of the pannicle; and C -- emerging panicle partially replaced by smut sori.

Infection of Sudan grass by S. reiliana does not always result in spore formation. In the absence of typical sorus development infected plants may bear inflorescences which are sterile and often peculiarly proliferated. Infected panicles were occasionally found which produced bizarre leafy structures as shown in Figure 2 (bottom). Induced phyllody as a secondary effect on host plants infected by S. reiliana has been observed in corn by Dana and Zundel (2), and in sorghum by Potter (12). Infection of Sudan grass by head smut is often manifested by this peculiar vegetative stimulation of the inflorescence which ultimately produces a phyllody condition

in the host panicle.

FIGURE 2. Head smut of Greenleaf Sudan grass showing: TOP -- fungus sporulation and shredding of the vascular tissues of diseased panicles; and BOTTOM -- vegetative proliferation or induced phyllody in panicles infected with Sphacelotheca reiliana.

HEAD SMUT OF GRAIN SORGHUM

In sorghums the head smut problem is attributed chiefly to the widespread planting of susceptible hybrids. For many years uniformly ripening standard varieties of grain sorghum were grown in California. These included such well-known varieties as Double Dwarf 38, Double Dwarf Yellow Sooner, Ryer 15, Norghum, and Reliance. The sporadic occurrence of head smut found in these standard varieties was considered of negligible importance. The discovery of cystoplasmic male sterility in sorghum has led to the development of many hybrid varieties. The beneficial consequence of sorghum hybrids was an increase in grain yields of 20 to 25 percent. Following in the wake of the profitable precedent set with hybrid corn, hybrid sorghums were widely accepted by growers in grain sorghum areas. In California the yields of 23 hybrids

FIGURE 3. Sporulation of Sphacelotheca reiliana on Greenleaf Sudan grass. A -- sporulation on host culms; B -- sorus at the base of a sterile panicle; and C -- sori covered by white peridia formed on Sudan grass foliage.

tested in many growing areas of the State under varying environmental conditions showed significant yield increases over old line varieties, according to Smeltzer et al. (15). One of these hybrid varieties, RS 610, outyielded the standard California varieties by 33 percent in 1957 and 28 percent in 1958. In 1958-59 hybrids accounted for more than one-half of the grain-sorghum acreage in the Sacramento Valley.

From 1956 through 1959 head smut has been collected in California on the following varieties: Ryer 15, Double Dwarf 38, Double Dwarf Yellow Sooner, Dwarf White Durra, Dwarf Yellow Milo, Norghum, Reliance, Chiltex, Leoti sorgo, and Sumac sorgo. Many sorghum hybrids have also been found infected with head smut as follows: Amak R10, DeKalb E56a, DeKalb F62a, NK 210, PAG 425S, PAG Exp. 2033, RS 590, RS 610, RS 650, T 601, T 660, Combine 7078, and male sterile Combine Kafir. Some of these varieties and hybrids have already been reported as susceptible to head smut by other workers (13, 14, 17). As the acreage of hybrid sorghums expands in California and their cultivation continues indefinitely, the head smut problem might eventually become even more accentuated.

CROSS-INOCULATION STUDIES

Cross-inoculation experiments were conducted to establish whether head smut which is prevalent on Sudan grass and the same fungus common on grain sorghum would cross-infect. It was important to determine whether the large reservoir of naturally perpetuated head smut in

volunteer Sudan grass was potentially a threat to grain sorghum production in the same area. Spore inocula from Greenleaf Sudan grass and Ryer 15 grain sorghum were obtained by making separate field collections of the disease on these two hosts. Smutted heads were individually ground up in a Wiley mill fitted with a coarse mesh screen. Inoculum from infected Sudan grass was mixed into a plot of field soil in December. Spores obtained from grain sorghum were incorporated into the soil of another field plot on the same day. A third area was staked out for use as an experimental control. All three plots were planted the following spring with a selected range of Sudan, sorgo, and sorghum varieties. The results of these field tests with soil-borne spore inocula are presented in Table 1.

Table 1. Reaction of selected varieties of Sudan grass, sorgo, and grain sorghum to two collections of head smut (Sphacelotheca reiliana).

Variety	Source of inoculum and infection percentages					
	Sudan grass smut		Grain sorghum smut		Controls - no smut	
	Culms (number)	Infected (percent)	Culms (number)	Infected (percent)	Culms (number)	Infected (percent)
Greenleaf Sudan	164	68.9	270	40.4	205	1.0
Sweet Sudan	199	57.3	246	32.5	204	0.0
Sudan 23	227	18.1	335	2.7	375	0.0
Tift Sudan	231	3.5	249	1.0	219	0.0
Piper Sudan	303	2.3	374	0.0	378	0.0
Lahoma Sudan	119	0.0	124	0.0	221	0.0
Leoti sorgo	52	92.3	104	66.4	69	0.0
Sumac sorgo	32	68.8	85	36.5	79	0.0
Ryer 15 sorghum	45	46.7	68	16.2	101	0.0
DD 38 sorghum	52	34.6	59	6.8	73	0.0

Two varieties of corn, Stowells Evergreen and Country Gentleman, reported by Mankin (11) as susceptible to the corn strain of S. reiliana were included in these tests. No infection of these varieties was obtained with either of the smuts used in this test.

DISCUSSION

Head smut has been known in the United States as a disease on sorghum since 1890 and on corn since 1895 (12, 13). Although the fungus is widely distributed on these crops the total losses from it have been small. Physiologic specialization in S. reiliana has also been established with one race principally limited to corn and another to sorghum (1, 11, 14). In general, Sudan grass has been considered only slightly susceptible and the sporadic reports of head smut on this host (3, 18) indicate a mycological interest rather than economic losses. In central California, however, the disease has become widespread in Sudan grass in several counties during 1957-59 and the economic losses suffered by certified seed growers are substantial. Losses in grain yields are also inflicted by the fungus as a consequence of the widespread cultivation of susceptible varieties of hybrid grain sorghums. The spread of the disease has no doubt been further enhanced by the use of smut-contaminated seed, especially of hybrids grown in areas where head smut has long been a problem.

Differences in reaction to head smut were noted among six varieties of Sudan grass studied (Table 1). In view of these differences the pedigrees of these varieties, as shown in Table 2, are of interest. Sudan 23, which is moderately susceptible, was developed by selection from Common Sudan grass. Each of the other five varieties have the highly smut-susceptible Leoti sorgo and Common Sudan grass in their parentage. From published information (4, 5, 6, 7, 8) regarding the development of these varieties, reaction to head smut was apparently not a factor in selection. Yet from these separate breeding programs came varieties Greenleaf and Sweet which are highly susceptible, Tift and Piper which show low infections, and Lahoma which is apparently immune to the collections of head smut used in this study. The reaction of these varieties to S. reiliana may thus depend on the particular plants of Common Sudan grass used in the breeding programs. It is evident, as suggested earlier by Stevens (16), that plant breeding programs may indeed complicate plant disease problems.

In addition to the cultivated crops of Sudan grass being subject to infection, the fungus is

Table 2. Analysis of the pedigrees of six Sudan grass varieties grown in California showing their geographic origin and parentage.

Variety	Place of origin	Pedigree of variety	Reference
Sudan 23	California (Released 1937)	Selection from Common Sudan	(5)
Tift	Tifton, Georgia (Released 1942)	LEOTI x Common Sudan	(4, 5, 7)
Sweet	Texas (SA-372) (Released 1943)	LEOTI x Common Sudan	(4, 5, 6)
Piper	Wisconsin (Released 1950)	TIFT x (Lines from Texas / Lines from Kansas)	(4, 5)
Greenleaf	Kansas (Released 1953)	LEOTI x Sudan intercross	(4, 5, 8)
Lahoma	Oklahoma (Released 1954)	LEOTI x Common Sudan	(4, 5)

also commonly found on volunteer plants of Sudan grass growing along roadsides, irrigation ditches, fence rows, in pastures, and occasionally in irrigated tomato fields. As an example, one such plant was found bearing 17 infected tillers and only 3 healthy panicles. Such smutted plants exposed to prevailing winds undoubtedly result in local dissemination of spore inoculum which facilitates soil infestation (12). A reservoir of smut inoculum may thus be naturally perpetuated from year to year in volunteer plants of susceptible Sudan grass varieties.

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INHERITANCE OF RESISTANCE OF STRAWBERRY TO THE COMMON RACE OF THE RED STELE ROOT ROT FUNGUS[1]

Gene Stembridge and D. H. Scott[2]

Abstract

Seedling strawberry progenies and asexually propagated plants of the varieties used as parents were grown in the greenhouse in soil infested with the common race of the red stele fungus. Evaluations of resistance were based on the percentage of the root system showing symptoms of red stele.

The results obtained indicated that resistance to red stele infection is partially dominant and is quantitatively inherited. Evidence for the independency of inheritance of the Aberdeen and Frith (Scottish) types of resistance was shown. Aberdeen-type resistance was transmitted to approximately one-third of the progeny when a resistant parent was crossed with a completely susceptible parent.

INTRODUCTION

The red stele root disease of strawberries, caused by Phytophthora fragariae, has become of major importance in strawberry-growing regions throughout the world since its discovery in Scotland about 1920.

Control of the disease by cultural practices or by chemical means has proved unsuccessful; the only practical method of control is the use of resistant varieties. Breeding programs to originate resistant varieties were initiated in Scotland in 1933, and in the United States by the United States Department of Agriculture in 1937.

Early surveys to find resistant material upon which to base breeding programs resulted in the discovery of two major sources of resistance. In Scotland, a selection believed to be the obscure variety Frith proved to be resistant (6), while the American variety Aberdeen was found to be resistant in the United States (1). These two sources of resistance were used extensively in previous breeding work.

The discovery of physiologic races of the red stele fungus pathogenic to varieties having the Aberdeen and Frith types of resistance (2, 4) has increased the importance of the sources of resistance in Fragaria virginiana and F. chiloensis. However, either the Aberdeen or the Frith type of resistance is effective against the common race of the red stele pathogen, and, as a result of previous breeding work, these two resistance types are available in combination with other desirable horticultural characteristics.

While few experiments have been conducted solely for the purpose of determining the inheritance of resistance, observations of seedling progenies grown as an integral part of breeding programs have shown certain general trends in the inheritance of resistance.

To equate past observations in exact terms of inheritance, particularly in regard to races of the fungus and sources of resistance, is impossible. Many of these observations were recorded before physiologic specialization of the fungus was recognized. Lack of uniformity and severity of infection in methods used to test resistance further complicate the situation. Nevertheless, certain general patterns of inheritance from the Aberdeen and Frith sources were determined.

Reid (5, 6) reported that Frith, when selfed or crossed with susceptible varieties, transmitted resistance varying from 36 to more than 50 percent of its offspring.

Progenies of Scottish selections having resistance derived from Frith were field tested in infested soil in the United States (3). Results indicated that resistance from these selections was transmitted to somewhat less than half of their progeny when a susceptible variety was used as the other parent.

Temple (10) reported that Aberdeen, when crossed with a susceptible variety, transmitted resistance to a high percentage of its progeny. Field tests by Jeffers et al. (3) substantiated this report by showing that approximately 60 percent of the seedlings from crosses involving Ab-

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erdeen and a susceptible variety were resistant to red stele.

Aberdeen was found highly resistant under Scottish conditions, and progenies from selfed Aberdeen often contained 60 to 80 percent resistant seedlings (6). This is a greater percentage of resistant seedlings than obtained under similar conditions by selfing Frith (6).

A generalization may be made from the data presented by Reid (6) and by Jeffers et al. (3) that Aberdeen-type parents transmit resistance to a greater proportion of their offspring than do Frith-type parents. A hypothesis that the Frith and Aberdeen types of resistance are independent may be drawn from the data of Scott et al. (8); plants carrying each type of resistance exhibited a different reaction to various races of the fungus. These workers (9) also found that selections from crosses of Aberdeen-type and Frith-type parents had varying degrees of these two types of resistance.

The purpose of the present paper is to present additional information regarding the heritability of red stele resistance from the Frith and Aberdeen sources.

MATERIALS AND METHODS

Seedling strawberry progenies and asexually propagated plants of the varieties used as parents were grown in the greenhouse in soil shown by previous tests to be infested with the common race of the red stele fungus (race A-1), but contamination from other races was a possibility. Plants having neither Aberdeen-type nor Frith-type resistance are not susceptible to this race of the pathogen (2).

Testing for red stele resistance was essentially the same as reported previously (7, 11). Metal benches 5 inches deep were equipped with valve-regulated bottom drainage and coated on the inside with a waterproof roofing compound. One inch of steam sterilized gravel was placed in the benches and covered with about 2 1/2 inches of infested soil. One and one-half inches of steam sterilized soil was added, bringing the soil level to within 1 inch of the top of the bench.

Separate randomized block designs were used for seedling progenies and asexually propagated plants of the parent varieties. The seedlings were set 1 1/2 inches apart in 32-plant plots. A block contained a plot of each progeny, and eight randomized blocks were used. Thus, 256 plants of each progeny were tested.

Plants of the parent varieties were spaced 3 inches apart in 3-plant plots. A block contained a plot of each variety, and four randomized blocks were used.

Seedlings were planted in October 1958, and parent plants were set in the benches in November 1958. The temperature was maintained at about 70° F until January 1959 and then lowered to 50° and the benches flooded twice each week.

Severe wilting, which is symptomatic of red stele infection, began to appear early in February 1959. Severely wilted plants were removed from the benches at weekly intervals during February and March, in order that the disease could be identified before secondary infection obscured the symptoms on the roots. The remaining plants were removed from the benches on March 26, 1959.

All plants were classified according to the severity of infection of the root systems based on visual observation irrespective of their time of removal from the benches. The plants were grouped in classes as follows: class 1, very severely infected plants with more than 75 percent of the root system infected; class 2, severely infected plants with 50 to 75 percent of the root system infected; class 3, moderately infected plants with more than 25 but less than 50 percent of the root system infected; class 4, slightly infected plants with less than 25 percent of the root system infected; class 5, plants with no visible sign of red stele infection.

Wilted plants which were removed at weekly intervals prior to the final inspection were very severely infected; all of these plants were placed in class 1. Classes 1, 2, and 3 were grouped together and classes 4 and 5 were combined, forming two categories: Susceptible and resistant.

A resistance rating system was also used for comparisons of resistance. This rating was calculated for each progeny and parent by multiplying the number of plants in each class by that class value and then dividing the sum of these products by the number of plants classified. A resistance rating of 1.000, therefore, indicates that all the plants were in class 1, and increasingly higher resistance ratings indicate increased proportions of plants in the higher classes.

The resistance ratings were used in the analysis of variance of the seedling progenies. A few of the seedlings died of undetermined causes prior to the classification of progenies. However, since the number of classified seedlings in each replication varied only slightly, unweighed analyses were used.

RESULTS

Resistance tests of the parent varieties showed that Gem and Midland were completely susceptible, while Sparkle, Surecrop, and Md-US-2389 were infected only in an occasional root tip (Table 1).

Table 1. Sources of resistance of parent strawberry plants and the relative resistance of parent varieties to race A-1 of the red stele fungus.

Parent variety or selection	Source of resistance	Number of plants in each resistance class[a]					Resistance rating
		1	2	3	4	5	
Midland	None	10	0	0	0	0	1.00
Gem	None	9	2	0	0	0	1.18
Surecrop	Aberdeen and Frith	0	0	0	9[b]	2	4.18
Md-US-2389	Aberdeen	0	0	0	7[b]	2	4.22
Sparkle	Aberdeen	0	0	0	7[b]	3	4.30

[a] A few plants died of undetermined causes and were not classified.

[b] Only a few root tips appeared diseased.

Table 2. Relative resistance of seedling strawberry progenies to race A-1 of the red stele fungus.

Progeny	Number plants examined[a]	Number of plants in each resistance class					Average resistance rating (8 replications)	Percent resistant seedlings[b]
		1	2	3	4	5		
Midland open-pollinated	245	232	12	0	1	0	1.07	0.41
Gem x Sparkle	231	44	47	54	84	2	2.79	37.23
Surecrop x Md-US-2389	238	14	12	37	159	16	3.64	73.53
L.S.D. .01 =	.21							
L.S.D. .05 =	.15							

[a] A few plants died of undetermined causes and were not classified.

[b] Plants in classes 4 and 5 were considered resistant.

The progenies showed marked differences in their degree of resistance to the common race of the red stele fungus (Table 2). An application of Duncan's multiple range test showed that highly significant differences exist between the resistance ratings of any two progenies, that is, all differences between progenies are highly significant.

Since Midland open-pollinated progeny contained only one resistant seedling, infection was considered to be uniform throughout the benches. The occurrence of a resistant seedling in this progeny was probably the result of either cross-pollination or an error in grouping the progenies.

DISCUSSION

The distribution of the seedling plants in the various infection classes indicates that genes for resistance are cumulative in their effect and that practically all degrees of resistance are to be expected in a progeny if resistance is present in the parentage. The cumulative nature of resistance is further evidenced by the increased resistance shown in the progeny having two re-

sistant parents as compared with that of the progeny having only one resistant parent.

The progeny from only susceptible parents (Midland open pollinated) produced only one resistant offspring, thus indicating that resistance is partially dominant as previously reported (7).

Sparkle, a variety having Aberdeen-type resistance, transmitted resistance to race A-1 to approximately one-third of its progeny when crossed with a susceptible variety. Parents having Frith-type resistance were reported by Scott et al. (7) to produce only 20 to 25 percent resistant seedlings when crossed with a susceptible variety. These workers utilized a physiologic race of the red stele fungus which is pathogenic to plants having only Aberdeen-type resistance, subsequently designated race A-3 (2).

A comparison of the results of these two tests gives additional indication that resistance to race A-1 from Aberdeen-type parents is transmitted to a greater proportion of a progeny than is resistance to race A-3 from Frith-type parents.

The present study also furnishes substantiating evidence for the independency of the Aberdeen and Frith types of resistance. The cross of two resistant plants yielded twice as many resistant seedlings as did the cross involving only one resistant parent. Chance segregation and recombination of genes would be expected to preclude the strictly additive effect of two resistant parents in regard to the percentage of resistant seedlings produced. However, one of the parents used in this cross (Surecrop) has multiple resistance and can be considered as having two effective complements of genes for resistance to race A-1 if the two types of resistance are considered independent. The large percentage of resistant seedlings obtained from this cross of two resistant varieties lends credence to the supposition regarding the independency of the Aberdeen and Frith types of resistance.

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CHEMICAL DIPS FOR THE CONTROL OF NEMATODES ON BARE ROOT NURSERY STOCK[1]

Lee Jenkins[2] and H. W. Guengerich

There is an increasing need for a satisfactory method of treating nematode-infested nursery stock before it is shipped to the customer. Damage to slightly infested trees or shrubs may be negligible when delivered to the customer. Detection of slight infestations is practically impossible where millions of trees and shrubs, many with innumerable small roots, are involved. These slightly infested plants may become seriously injured soon after planting, due to a build-up of the initial nematode infestation. An effective treatment would provide a means of insuring nematode-free nursery stock. Systematic use of such a treatment would prevent the spread of nematodes in shipments of trees and shrubs from the nursery.

Renninger, Coffey and Sokoloff[3] reduced Radopholus similis, the burrowing nematode, 94.9 percent on citrus roots by treating the soil with a soluble hydrogenated fish oil.

Tests to explore the possibilities of using a chemical as a dip on bare root nursery stock were started by the authors in 1958. Experimental materials from the Union Oil Company of Brea, California, known as G6822, G1676 and 279, were tested. The American Cyanamid Company furnished an emulsifiable organic phosphate experimental nematocide 18133 (O, O-diethyl O-2-pyrazinyl phosphorothioate) and Boris Sokoloff of the Southern Bio-Research at Lakeland, Florida furnished hydrogenated fish oil for our tests. Of those materials tested, the 18133 seemed to be the most promising when both plant tolerance and nematode control are considered. The treatments were directed for the most part toward the control of root-knot nematodes, Meloidogyne sp.

MATERIALS AND METHODS

The materials Union Oil G6822, G1676, 279 and American Cyanamid 18133 were tested in 1958. Weigela hybrida (var. Eva Rathke) plants that were heavily infested with root-knot nematodes were taken from storage on March 10 and dipped in Union Oil Company's formulation G1676 diluted at the rate of 14 grams of the 25 percent material per gallon of water. Union Oil G6822 was diluted at the same rate as the G1676. Five Weigela plants were used for each treatment. The whole plants were soaked for 5 minutes, then removed and drained. Five plants were soaked in water for 5 minutes as a check. After draining, the plants were permitted to dry for 1 hour and then were packaged. Forty-eight hours later all the plants were unpacked, dipped in activated charcoal (1 pound in 20 gallons of water), and repacked. The activated charcoal was used to reduce the objectionable odor of the chemicals used as a dip. All plants were placed in 6 1/2-inch plots of steam sterilized soil on March 14 and put in a greenhouse where they were grown for 3 months.

The material Union Oil Co. 279 was diluted with water at the rate of 2 2/3 ounces in 15 gallons of water.

American Cyanamid 18133 in the 50 percent emulsifiable form was used at three rates as follows: 9 ounces of 18133 in 15 gallons of water; 4 1/2 ounces of 18133 in 15 gallons of water; and 2 1/4 ounces of 18133 in 15 gallons of water.

The plants were soaked for 5 minutes in the above concentrations. No activated charcoal was used following treatment.

On April 10 of 1959 American Cyanamid 18133 and the hydrogenated fish oil furnished by Boris Sokoloff were used. The 18133 emulsifiable was used at the rate of 4 1/2 ounces of the 50 percent in 15 gallons of water at a temperature of 52° F. The plants were soaked for 15 minutes.

The hydrogenated fish oil was dissolved in rain water to avoid forming a precipitate when tap water was used. The plants were soaked for 15 minutes at a temperature of 115°. The check plants were dipped for 15 minutes in water at 52°. The higher temperature was used for the hydrogenated fish oil because it was difficult to dissolve.

Included in this test were Talisman rose, Weigela hybrida (var. Eva Rathke), and Fire thorn. These plants were used because they were heavily infested with root-knot nematodes.

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[1] Contribution from Missouri Agricultural Experiment Station, Journal Series No. 2059. Approved by Director.

[2] Department of Entomology.

[3] Renninger, George, John Coffey, and Boris Sokoloff. 1958. Effect of hydrogenated fish oils on citrus-tree destroying nematodes. Plant Disease Reptr. 42: 1057 - 1065.

Table 1. Results of chemical dips on bare root nursery stock for the control of root-knot nematodes.

Date Treated	Chemical and Rate	Minutes Treated	No. of Plants	Condition of Plants When Checked	No. of Female Root-Knot Nematodes Observed
April 30, 1958	American Cyanamid 18133 9 oz. 50% in 15 gal.	5	5 Weigela	All dead	0
April 30, 1958	American Cyanamid 18133 4-1/2 oz. 50% in 15 gal.	5	5 Weigela	3 dead - 2 alive	0
April 30, 1958	American Cyanamid 18133 2-1/4 oz. 50% in 15 gal.	5	5 Weigela	3 dead - 2 alive	1
April 30, 1958	Union Oil 279 2-2/3 oz. in 15 gal.	5	5 Weigela	5 dead	0
March 10, 1958	Union Oil G6822 14 grams 25% per gal.	5	5 Weigela	4 dead - 1 alive	Numerous
March 10, 1958	Union Oil G1676 14 grams 25% per gal.	5	5 Weigela	5 dead	Numerous
March 10, 1958	Check - Water Only	5	5 Weigela	2 dead - 3 alive	Numerous
April 11, 1959	Hydrogenated Fish Oil 1%	15	5 Weigela	5 alive	1 nematode on each of 2 plants
April 11, 1959	American Cyanamid 18133 4-1/2 oz. 50% in 15 gal.	15	5 Weigela	5 alive	4 nematodes on one small root
April 11, 1959	Water Only	15	5 Weigela	2 dead - 3 alive	Numerous
April 11, 1959	Water Only	15	2 Fire Thorn	2 dead	--[a]
April 11, 1959	Hydrogenated Fish Oil 1%	15	2 Fire Thorn	2 dead	--[a]
April 11, 1959	Hydrogenated Fish Oil 1%	15	2 Talisman Rose	1 dead - 1 nearly dead	0
April 11, 1959	American Cyanamid 18133 4-1/2 oz. 50% in 15 gal.	15	2 Talisman Rose	1 dead - 1 alive	0
April 11, 1959	Water Only	15	2 Talisman Rose	1 dead - 1 alive	0

[a]The Fire thorns died soon after planting and were not in condition favorable for nematode development.

The plants were treated April 10 and planted in sterilized soil in 8-inch plots on April 13. They were left in these plots until June 16, and then were examined for the presence of female root-knot nematodes in the roots. All roots showing evidence of damage by root-knot nematodes were examined under the microscope using a dissecting needle to tear the root tissues apart and expose the nematodes.

In addition to the plant material from Missouri, four budded Mazzard cherries from California were furnished by Paul S. Jorgensen of Stockton, California. These cherry trees were infested with the lesion nematode Pratylenchus penetrans. Two of these trees were soaked in a solution of 4 1/2 ounces of 50 percent American Cyanamid 18133 for 15 minutes and two were soaked in a 1 percent concentration of hydrogenated fish oil for 15 minutes. After treating, the trees were returned to Dr. Jorgensen, who grew them for 2 months in moist "sponge rok." There were no untreated checks in this experiment.

RESULTS

The Mazzard cherries checked by Dr. Jorgensen had no nematodes present on the roots after growing for 60 days in "sponge rok" in either of the treatments using American Cyanamid 18133 or hydrogenated fish oil. The hydrogenated fish oil caused damage to the roots and an area about 2 inches above and 2 inches below the soil line. The Mazzard cherry trees treated with American Cyanamid 18133 had good root growth and fair top growth.

Table 1 gives the results of the tests in 1958 and 1959.

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HOST RANGES AND LATENT CARRIERS OF LAMBERT MOTTLE IN PRUNUS SPECIES[1]

I. K. Mills and M. M. Afanasiev

Abstract

The following varieties of cherries and other species of Prunus were inoculated with Lambert mottle virus: Lambert, Bing, Royal Ann, Deacon, Black Tartarian, Black Republican, Van, Stark Gold, Starking Gold Giant, Seneca, Mazzard, Mahaleb, Shiro-fugen, Montmorency, chokecherry, Italian prune, and Lovell peach. The symptoms of this disease appeared only on Lambert, Seneca, and Starking Gold Giant cherries. When all inoculated trees were indexed on Lambert all of them with the exception of Shiro-fugen, Montmorency, chokecherry, and Italian prune produced a positive reaction.

Lambert mottle has also been recovered from symptomless trees of Stark Gold and Black Tartarian trees grown in commercial orchards.

Symptoms of Lambert mottle virosis were first reported to occur on Lambert cherry, while Bing and Royal Ann varieties were found to be symptomless carriers (3). Typical foliage symptoms of the disease resulting from natural spread were also observed on the Peerless variety (1, 2).

Experiments were started in 1956 to investigate the behavior and effects of Lambert mottle upon various varieties of sweet cherries and other species of Prunus. Buds taken from a single Lambert tree showing severe symptoms of Lambert mottle were placed on three trees of each of the following kinds: Lambert, Bing, Royal Ann, Deacon, Black Tartarian, Black Republican, Van, Stark Gold, Starking Gold Giant, Seneca, Mazzard, Mahaleb, Shiro-fugen, Montmorency, chokecherry, and Italian prune. In 1957 and subsequently, leaf symptoms of this disease appeared only on Lambert, Seneca, and Starking Gold Giant cherries. All inoculated trees were indexed on Lambert to determine if the symptomless varieties carried latent Lambert mottle. Positive reactions in the Lambert test trees were obtained with all the above-mentioned trees with the exception of Shiro-fugen, Montmorency, Italian prune, and chokecherry.

Lovell peach trees inoculated from the same inoculum source in 1957 produced Lambert mottle when indexed on Lambert the following year.

Lambert trees infected with Lambert mottle may provide the major source of inoculum for the spread of this disease in orchards. However, since removal of infected trees is the only known method of control of the disease, it is important to determine whether symptomless but susceptible cherry varieties may serve as a reservoir for the virus. The "natural" occurrence of Lambert mottle on cherry varieties other than Lambert and Peerless was investigated by bud-inoculating Lambert trees from a limited number of Bing, May Duke, Stark Gold, Black Tartarian, Centennial, Windsor, Deacon, and Mazzard seedling trees growing in three different commercial orchards where Lambert mottle occurred. Lambert trees inoculated from these sources were examined during the following summer and it was found that Stark Gold and Black Tartarian trees produced a positive reaction for Lambert mottle. Those trees which produced a positive response were re-indexed and again produced Lambert mottle in test trees.

These results show that many varieties of cherries, commercial and pollinizers as well as the common rootstock species, carried Lambert mottle with or without symptom expression. Further, the fact that Lambert mottle has been recovered from symptomless pollinizers in commercial orchards indicates that effective control of the virosis probably cannot be obtained by eradicating only symptom-bearing trees.

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EVALUATION OF APPLICATION METHODS FOR APPLYING 1,2-DIBROMO-3-CHLOROPROPANE FOR CONTROL OF ROOT KNOT[1]

J. M. Good and A. E. Steele[2]

Summary

A comparison of several methods of applying 1,2-dibromo-3-chloropropane soil fumigant for control of root-knot nematodes indicated that liquid and granular formulations gave adequate control if the chemical, regardless of formulation or method of application used, was placed 6 inches or more under the soil surface. A granular formulation applied to the soil surface and worked in with a disk harrow was not as effective as other methods of application.

Formulations of 1,2-dibromo-3-chloropropane (DBCP) adsorbed on Attaclay granules were placed on the market 3 years ago in Georgia, and have been used in increasingly larger amounts on soil for a number of kinds of crop plants. It soon became apparent that farmers were applying this material in many different ways, and sometimes using methods of questionable merit, yet in most cases nematode control appeared satisfactory. These methods included spreading DBCP-impregnated-granules on the soil surface with fertilizer spreaders and working the material into the soil with disk harrows, turning-plows, or tillage-disk plows; mixing the granular formulation with fertilizer and putting it in a band under the planting row 6 to 8 inches deep for large-seed crops or 2 to 3 inches deep for some small-seed crops; placement of the granules 6 to 8 inches deep with small grain drills; and side dressing applications of granules to row crops and perennials. Most of the above methods have not been evaluated under experimental conditions whereby their relative efficacy could be compared with standard techniques of chisel injection of liquid DBCP and other nematocides.

The use of granular or solid carriers for ethylene dibromide (EDB) and DD Mixture has been investigated by a number of workers, who have suggested several advantages for their use (9, 10, 11, 12, 13). The mechanical mixing characteristics of several types of equipment for incorporation of solid materials into the soil have been evaluated by Morrison and Crowell (6), Grainger (5), and Newhall and Gunkel (8), who found that disking and rotary tillage tools were generally unsatisfactory for this purpose, especially if materials are to be incorporated below a depth of 5 to 6 inches. In these studies iron filings, radioactive iodine, and seed, respectively, were used to evaluate the mechanical mixing characteristics of the various tools. These studies do not fully evaluate the methods as they might apply to volatile chemicals that will diffuse through the soil, possibly reducing the need for thorough mixing. Gilpatrick et al. (2) reported that DBCP could be effectively used at shallower depths than either DD Mixture or EDB because of its lower volatility. Granular formulations of DBCP were as effective as liquid formulations at depths of 4 to 8 inches but were less effective at a 2-inch depth. At higher than usual dosage rates, Good and Steele (3) reported that a granular formulation of DBCP when incorporated into the soil with a disk harrow gave satisfactory control of Meloidogyne incognita on tomatoes grown for fruit production, but they did not obtain desirable root-knot free transplants. In another experiment (4) they reported preplanting chisel injection of DBCP gave better control of Pratylenchus brachyurus on peanuts than either a side dressing application of liquid DBCP injected with chisels or solid preplanting application of a granular formulation of DBCP that was worked into the soil with a disk harrow. In New Mexico, Morton (7) reported that in-the-row applications of granular DBCP gave adequate root-knot nematode control on cotton, but that chisel injection of liquid DBCP gave somewhat better control than the granular formulation; all treatments were superior to unfumigated controls. Working in North Carolina, Cooper et al. (1) found that a granular formulation of DBCP applied as preplanting applications gave better control of sting nematode than post-planting side dressing applications on peanuts, corn, soybeans, and cotton.

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[1]Cooperative investigation of the Crops Research Division, Agricultural Research Service, United States Department of Agriculture and the University of Georgia College of Agriculture, Agricultural Experiment Stations.

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MATERIALS AND METHODS

The soil of the experimental field was a Tifton loamy sand and was lightly-to-moderately infested with Meloidogyne incognita incognita (Kofoid and White) Chitwood, 1949. The test was arranged in randomized blocks with six replications of eight soil treatments. Each plot had two 50-foot rows spaced 38 inches apart. One row of each plot was planted to the Rutgers variety of tomatoes (Lycopersicum esculentum) and the other to Summer Crook Neck squash (Cucurbita pepo var. melopepo).

Six methods of applying DBCP[3] were compared with unfumigated controls and a standard row application of DD Mixture[4]. Row treatments with DBCP consisted of: 1) emulsible solution injected with constant gravity-flow equipment to a depth of 8 to 10 inches, the injection rows being listed on with disk hiller plows (Fig. 1); 2) DBCP impregnated granules distributed in the fertilizer furrow by means of a granular insecticide attachment (Fig. 2); and 3) a premixed blend of fertilizer and DBCP-impregnated granules distributed as a band placement under the planting row with standard fertilizer distributors (Fig. 2). Solid, or over-all, application methods consisted of: 1) emulsible solution injected on 12-inch centers to a depth of 8 to 10 inches with constant gravity-flow equipment (Fig. 3); 2) DBCP-impregnated granules spread evenly over the soil surface and cut-in with a disk harrow; and 3) placement of DBCP granules 6 to 8 inches deep on 12-inch centers with a modified small grain drill (also fertilizer spreader) having fertilizer spouts and points added (Fig. 4). All solid fumigation treatments were sealed with a drag to prevent excessive, rapid loss of the nematocide. The equipment used in this experiment was tractor-drawn and available on local markets.

The soil fumigant DD Mixture, which served as a standard control, was applied as a pre-planting in-the-row treatment at 12.6 gallons per acre. Twenty-five days after the DD treatment the beds were opened for fertilization. On the same day the various DBCP treatments were put out. The granular DBCP formulation was 17.3 percent by weight technical material adsorbed on Attaclay granules while the emulsible concentrate formulation was 70.3 percent by weight technical material. The latter formulation was diluted 1:9 with water to facilitate application. Solid applications were equivalent to 1.5 gallons per acre of technical DBCP (150 pounds/acre of 17.3 percent granules, or 3 gallons/acre of 70.3 percent emulsible concentrate). Row applications were equivalent to 1.75 quarts per acre of technical DBCP (43 pounds/acre of 17.3 percent granules, or 3.5 quarts/acre of 70.3 percent emulsible concentrate). Nematocide dosages for row treatments are based on a row width of 38 inches.

Squash seed and root-knot free tomato transplants were planted on April 30, 1958, five days after application of DBCP. A 2-foot spacing was used between hills for both tomatoes and squash. Every other tomato plant was dug on June 10 in order to get a root-knot index early in the season. After the last harvest tomato and squash plants were dug and indexed for root knot on July 10, 1958. An index system of 0-4 (0, no galling, to 4, severe galling) was used for indexing individual plants for severity of root-knot infection.

RESULTS AND DISCUSSION

Root-knot indices for both tomato and squash test plants indicate that all methods of applying DBCP and DD Mixture significantly reduced root-knot nematode infection (Table 1). Except for the disk harrow method of incorporating the granular formulation of DBCP, solid applications of this chemical gave somewhat better root knot control than comparable row applications of DBCP and DD Mixture. All row applications of DBCP gave better root knot control than DD row treatments. In the row treatments liquid injection of DBCP was superior to applying granules in the fertilizer furrow or mixed with the fertilizer. In the solid treatments, where the granules were placed at a depth of 6 to 8 inches, control was equal to that obtained with the liquid formulation.

Previously published information and this data indicate that when DBCP is spread on the soil surface and worked in with a disk harrow the resulting nematode control is not always satisfactory, unless higer than normal dosage rates are used. In this experiment where the solid, or over-all, application rate was one-half higher than customarily used the disk harrow method was not equal to other solid application methods or to in-the-row applications of DBCP. This less effective control was probably attributable to loss of the fumes from that portion of the chemical that was mixed in the upper 2 to 3 inches of soil. It has long been known that it is difficult to produce a killing concentration of fumes in this part of the soil. The disk harrowing method of applying DBCP would probably be even less effective on heavier soils because of difficulties in incorporating the material. Granular formulations of DBCP if placed

[3]Technical material contains 97 percent by weight 1,2-dibromo-3-chloropropane.
[4]About 50 percent by weight 1,3-dichloropropene and 50 percent 1,2-dichloropropane.

FIGURE 1. Constant gravity-flow fumigation equipment for in-the-row treatment designed for handling two rows at a time.

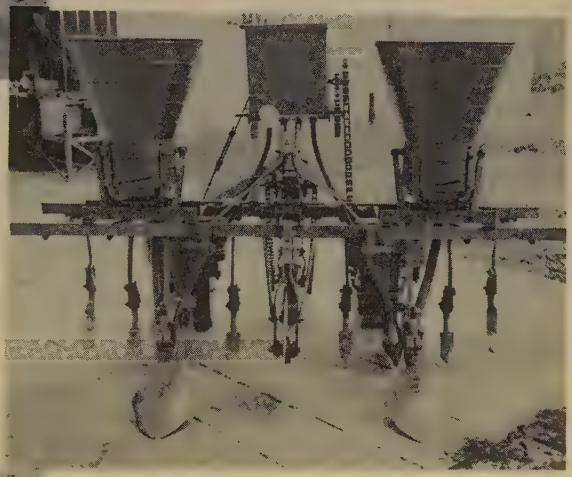

FIGURE 2. In-the-row application equipment for granular nematocides. Granular insecticide applicator (center) delivers granules through spouts into furrow behind opening plows. Premixed fertilizer-nematocide mixture can be applied in the planting row with standard fertilizer distributors (in this case for two rows).

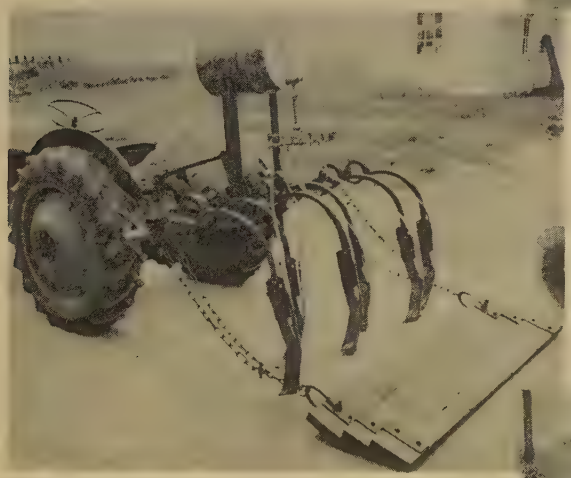

FIGURE 3. Constant gravity-flow solid fumigation equipment equipped with chisels for applying liquid fumigants.

FIGURE 4. Small grain drill (or fertilizer spreader) adapted for solid application of granular nematocides by adding fertilizer spouts and points.

Table 1. Effect of nematocide application methods on control of root knot on tomato and squash plants.

Application Methods	Average root knot indices[a] for six replications		
	Tomatoes		Squash
	June 10	July 23	July 23
DBCP Solid Treatments			
liquid, chisel	0.14	0.07	0.31
granular, applicator	0.03	0.05	0.32
granular, disk harrow	1.37	1.37	2.13
DBCP Row Treatments			
liquid, chisel	0.25	0.30	0.72
granular, applicator	0.91	1.22	1.53
granular, in fertilizer	1.05	1.45	1.49
DD Mixture, Row Treatments			
liquid, chisel	1.56	1.97	2.07
Unfumigated controls	2.16	2.37	3.07
L. S. D. .05	0.80	0.73	0.60
L. S. D. .01	1.07		0.80

[a]Method of deriving root-knot indices is described in the text.

deep enough in the soil are as effective as chisel application of liquid. Such formulations have practical farm value because of the ease of calibration and handling, reduced application costs, and the inherent appeal these formulations have for many growers. However, there was some loss of efficacy in this experiment when granular formulations of DBCP were mixed with fertilizer or applied separately on the fertilizer furrow. Nematocides are most efficient when applied at a depth of 6 to 10 inches; fertilizers are seldom placed as deep.

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INFLUENCE OF GERMINATING SEEDS OF SUGAR BEET (BETA VULGARIS) ON EMERGENCE OF LARVAE FROM CYSTS OF THE SUGAR-BEET NEMATODE (HETERODERA SCHACHTII)[1]

A. Morgan Golden and Thelma Shafer[2]

Summary

A treatment solution prepared from sugar-beet seeds germinated for as long as 4 days stimulated emergence of larvae from cysts of the sugar-beet nematode. Another solution, similarly prepared from seeds germinated for 5 days, produced an even more pronounced effect on larval emergence, approaching that of root diffusate of sugar beet. Apparently germinating seeds of sugar beet produce a substance or substances which tend to stimulate emergence of larvae from H. schachtii cysts.

The stimulatory effect of root diffusate of sugar beet (Beta vulgaris) upon the emergence of larvae from cysts of the sugar-beet nematode (Heterodera schachtii Schmidt) was demonstrated by Baunacke (1) over 35 years ago. Recently Golden (2) reported that leaf diffusate of sugar beet, although not as effective as beet root diffusate, tended to stimulate emergence of larvae from H. schachtii cysts.

The present preliminary work was conducted to determine whether germinating seeds of sugar beet produced an effective "hatching factor," as indicated by subsequent emergence of larvae from cysts of the sugar-beet nematode.

MATERIALS AND METHODS

Three hundred untreated seeds (seed "balls") of sugar beet were rinsed for 5 minutes in 400 ml of tap water. After removal from the water, which was kept, the seeds were equally divided into 15 groups of 20 seeds each. Each group of 20 seeds was immediately placed in a small glass jar containing two pieces of well-moistened filter paper on the bottom. The jars were then loosely covered and placed in a dark, aerated cabinet at room temperature of about 75° F in the day and slightly cooler at night. At regular intervals for 5 days, the following procedure was used: three jars, containing a total of 60 seeds, were taken from the cabinet and the seeds removed. These seeds were then rinsed for about 5 seconds in a small amount of water (this being saved) and all emerged radicles were measured and counted, after which these seeds were discarded. The filter paper was removed from the three jars and soaked for 30 minutes in 200 ml of water to remove any "hatching factor," before it also was discarded. After rinsing the jars with a small amount of water, this rinse water plus the rinse water from the 60 seeds and the 200 ml from the filter paper were made to a volume of 400 ml with other tap water. After being filtered, this was placed in the refrigerator (34° F) and was used as one treatment solution throughout the 6-weeks larval emergence test. Treatment solution B (Table 1) was obtained in this manner 1 day after initiation of the test; treatment solution C, 2 days afterwards; and so forth. Treatment solution A was the filtered 400 ml of water used originally to rinse the 300 seeds.

The methods and techniques for obtaining cysts, beet-root diffusate, and conducting the test were the same as previously used by Golden (3) in other larval emergence tests with the sugar-beet nematode.

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[1] Cooperative investigations of the Crops Research Division, Agricultural Research Service, United States Department of Agriculture and the Beet Sugar Development Foundation.

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Table 1. Emergence of larvae from H. schachtii cysts during 6-week test as influenced by germinating sugar-beet seeds.

Treatment solutions[a]	Time and percent seed germination	Total length of radicles (mm)	Emerged larvae: Total	Emerged larvae: Average per cyst
A (Seed rinse water)		--	6,686	41.8
B	1 day - 0%	0	8,945	55.9
C	2 days - 8%	5.0	7,687	48.0
D	3 days - 18%	75.0	7,037	43.9
E	4 days - 51%	360.0	12,483	78.0
F	5 days -- 55%	538.0	18,573	116.1
Beet Root diffusate	----------	-----	24,268	151.6

[a] For each treatment solution a total of 160 selected cysts divided into four replications of 40 cysts each were used.

RESULTS AND DISCUSSION

The treatment solutions prepared from beet seeds germinated for as long as 3 days had no evident stimulatory effect on larval emergence although a limited amount of seed germination and radicle growth had occurred (Table 1). This might have resulted, in part at least, from too much dilution of the "hatching factor," if present, during the preparation of the treatment solutions. The solution made from the seeds germinated for 4 days, however, produced a definite stimulatory effect on larval emergence; and the effect of the solution from seeds germinated for 5 days was even more pronounced, about three-fourths that of root diffusate of sugar beet.

The results indicate that germinating seeds of sugar beet produce some substance or substances which is effective in stimulating emergence of larvae from cysts of the sugar-beet nematode. In efforts to isolate the "hatching factor," germinating beet seeds in large quantities might provide a good source for the stimulatory material, presumably much freer of contaminating chemicals than is the usual root diffusate of sugar beet, especially if improved methods and sterile conditions during seed germination are used.

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VIABILITY AND PATHOGENICITY OF STORED HELMINTHOSPORIUM SOROKINIANUM CONIDIA[1]

R. G. Timian[2]

Summary

Helminthosporium sorokinianum Sacc. conidia were stored up to 42 months at 4° C without significant decrease in viability or pathogenicity. Several methods of storage, including storage over $CaCl_2$, lyophilizing, and mixing the spores with talc, were equally good as determined by spore germination and by pathogenicity on resistant and susceptible barley varieties.

INTRODUCTION

It is well known that Helminthosporium sorokinianum Sacc. (H. sativum), the cause of spot blotch of barley, is extremely variable in culture. Christensen (3, 4, 5) has shown that frequency of mutations in culture is high. Christensen and Davies (6) reported a mutation rate of 1:2900 in a monospore isolate after it had been passed through Marquis wheat ten times. Such a mutation rate can be very important in studies relative to the inheritance of resistance to the organism and in the development of resistant barley varieties. Since such studies commonly involve testing several generations of plant material over a period of years, pathogenic stability of the test organism is important. Any technique which will minimize the possibility of a change in pathogenicity is extremely advantageous. Decreasing the number of spore generations between isolation and inoculation of host plants by storage of the inoculum offers a possible means of lessening the number of mutations that may occur.

In 1956 Brandt (1) showed that there was no decrease in viability or loss of pathogenicity of conidia of H. sorokinianum after storage in a spore-talc mixture at 4° C for 3 months. Chinn and Ledingham (2) reported no decline in the survival of conidia of H. sorokinianum kept in dry soil (18 percent of moisture holding capacity) for 9 months. They reported 95 percent germination of conidia in dry soil with decreasing viability as moisture increased in the soil up to saturation.

The purpose of the present study was to determine whether conidia of H. sorokinianum could be stored for a relatively long period without significant losses in viability or pathogenicity.

MATERIALS AND METHODS

Sporulating cultures of H. sorokinianum isolated from barley grown at Fargo, North Dakota were grown at room temperature on potato-glucose agar in Petri dishes. When the cultures were approximately 2 weeks old, the Petri dish covers were removed and the agar allowed to harden. Cultures were then scraped with a scalpel and the mycelium removed from the collected mycelium-spore mass by sifting the spores through a single layer of cheesecloth. In this way a spore collection relatively free of mycelium was obtained. Harvested conidia were allowed to dry an additional 2 or 3 days at room temperature before being stored at 4° C in the following ways:

1. Lyophilized[3] with $CaCl_2$ before storage.
2. Mixed with talc in a 1-100 ratio and stored in a stoppered tube.
3. Stored in a stoppered tube.
4. Stored over $CaCl_2$ in a stoppered tube.

Post storage treatments of the conidia were as follows: Lyophilized conidia were allowed to hydrolize in unstoppered tubes at 4° C for 24 hours and were then mixed with talc and allowed

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[1] Cooperative investigation of the Crops Research Division, Agricultural Research Service, United States Department of Agriculture, and the North Dakota Agricultural Experiment Station, Fargo.

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[3] Lyophilization as discussed in this paper consisted of placing conidia in the bottom of a serological tube, along with a small quantity of $CaCl_2$ separated from the conidia with cotton. The tube was then evacuated at 15 pounds for 2 minutes at room temperature and sealed.

Table 1. The effect of different storage methods on the viability (percent germination) and pathogenicity (as determined by the reaction of six barley varieties) of H. sorokinianum conidia.

Treatment number	Storage condition at 4° C	Length of storage (months)	Conidia germination (percent)	Disease reaction[a]					
				Traill	Kindred	Ped. 38	U.M. 570	Modjo	N.D. B112
1	1-100 spore talc mixture	21	97	5	5	5	4-5	2	3
2	spores over $CaCl_2$ in stoppered tube	21	79	5	5	5	4-5	2	3
3	spores over $CaCl_2$ in stoppered tube	42	95	4	3	4	4	2	3
4	spores in stoppered tube	42	90	2	2-3	2-3	2-3	1	1
5	lyophilized spores	21	91	4	4	3-4	4	3	3-4
6	lyophilized spores	42	98	5	5	5	4-5	2	1
7	fresh spores[b]	0	82	5	5	5	4-5	3	3

[a] Disease reactions are given on a 1 to 5 basis, 1 being resistant and 5 being susceptible.

[b] Grown on potato-glucose agar inoculated with conidia from a culture of H. sorokinianum maintained by periodic transfers.

to stand overnight before being used. Conidia from the stoppered tubes with and without $CaCl_2$ were also mixed with talc and allowed to stand overnight before being used.

Germination counts were made as follows: Conidia-talc mixtures from the different storage treatments were dusted on potato-glucose agar in Petri dishes with a powder blower and incubated at room temperature. Counts were made periodically beginning 6 hours after the medium was seeded. The final count was made after 12 hours of incubation. The viability of conidia stored under different conditions was determined by calculating the percent of conidia germinated in microscope fields (215X) selected at random throughout the conidia-seeded agar plate. Conidia counts were made in a minimum of eight microscope fields per treatment, and a minimum of 100 conidia were observed for each treatment.

In order to determine the pathogenicity of stored conidia, barley plants in the 3-leaf stage were inoculated with the conidia-talc mixture from each of the storage treatments. The inoculum was applied with a powder duster. Prior to inoculation the plants were moistened with water to which a small amount of spreader (Tween 80) had been added. Following inoculation the plants were again moistened, incubated at 22° C for 40 hours in moist chambers, and then placed in a greenhouse maintained near 23°. Seven days after inoculation the disease reactions of the plants were read on a 1 to 5 (resistant to susceptible) basis.

RESULTS AND DISCUSSION

There was no significant loss in viability of H. sorokinianum conidia as determined by germination counts, regardless of the storage method. The range in percent germination was from 79 for conidia stored over $CaCl_2$ in a stoppered tube for 21 months to 98 for lyophilized conidia stored for 42 months (Table 1). Germination of freshly harvested conidia from a culture maintained by periodic transfers was 82 percent. Subsequent germination counts of conidia within treatments showed that the variation in percent germination observed could be expected.

There were only slight variations in the disease reactions of the six barley varieties inoculated with H. sorokinianum conidia stored under the different conditions (Table 1). The susceptible varieties Traill, Kindred, Pedigree 38, and U.M. 570 were susceptible regardless of the method of spore storage. All varieties were scored with a low disease reaction when inoculated with conidia stored in a stoppered tube for 42 months. The supply of conidia stored under this condition was not adequate to permit a second test. The disease reactions were low on plants inoculated with conidia stored over $CaCl_2$ in a stoppered tube for 42 months, but a second test showed no apparent loss in pathogenicity; a score of 5 was obtained on all four susceptible varieties, and the resistant varieties Modjo and N.D. B112 were scored 3-4 and 3, respectively.

The results of these studies indicate that conidia of H. sorokinianum may be stored at 4° C under reasonably dry conditions for at least 3 years without significant losses either in viability or pathogenicity. This fact should be very helpful to anyone studying the inheritance of resistance to the organism. It should also facilitate the inoculation of disease nurseries, since the inoculum can be produced months in advance of inoculation.

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A NEW STRAIN OF COMMON BEAN MOSAIC IN IDAHO[1]

Leslie L. Dean[2] and V. E. Wilson[3]

Summary

A previously unreported strain of common bean-mosaic virus infecting Great Northern UI-123 beans was discovered in Idaho in 1954. Symptoms incited by the virus strain were not sufficiently different from those incited by the type strain of bean virus 1 to permit distinction by observation. However, the differential infectivity among bean varieties resistant to known strains of common bean mosaic permitted distinction of the strain recovered from Great Northern UI-123. The virus infected all bean varieties tested which carried recessive resistance to the type strain of bean virus 1. Bean varieties carrying dominant resistance to the type strain of bean virus 1 were either resistant or susceptible to the newly found strain. The virus strain infecting Great Northern UI-123 was seed-borne in susceptible bean varieties.

The bean (Phaseolus vulgaris) variety Great Northern UI-123 (GN UI-123) is immune (4) from bean virus 1 (Marmor phaseoli Holmes) and from the variant strain reported from New York (6) and Idaho (2). Two distinct modes of inheritance of factors controlling resistance to the type strain of common bean mosaic have been demonstrated by Ali (1). Resistance derived from Corbett Refugee is inherited in a dominant manner, whereas that from Robust is recessive. Resistance of Great Northern bean varieties to the type strain of common bean-mosaic virus is apparently identical to that of Robust.

Plants of GN UI-123 bean with severe symptoms of mosaic were found in 1954. These symptoms were indistinguishable from those incited by bean virus 1 on susceptible varieties. Affected bean plants were distributed over a field of GN UI-123 growing at the Twin Falls Branch Agricultural Experiment Station, Kimberly, Idaho.

Inasmuch as recessive immunity from the type strain of common bean mosaic had been widely incorporated into dry bean varieties, steps were taken to ascertain whether a different strain of bean mosaic had become established or whether an inadvertent seed mixture had occurred.

A second planting of GN UI-123 beans from the same seed source was examined for evidence of common bean-mosaic-infected plants, but none was found.

METHODS

Mosaic-infected GN UI-123 plants were marked and numbered in the field. Several leaves were removed from each plant, and a series of mosaic-free GN UI-123 seedlings grown in a greenhouse were inoculated from each field plant that showed mosaic symptoms. A modification of the leaf abrasion method of inoculation described by Pierce (5) was used. The virus from individual plants was maintained in the greenhouse by successive mechanical inoculation to GN UI-123 seedlings.

Seed from each GN UI-123 plant marked in the field, as well as seed produced by mechanically inoculated plants grown in the greenhouse, was harvested individually. This seed was grown in the greenhouse to determine whether the virus was transmitted through the seed.

A series of bean varieties (Table 1) was inoculated with the new virus from a seed-borne source to ascertain whether resistant plants were present among popular varieties and also to attempt to distinguish this apparently new virus from those already recognized.

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[1] Research Paper No. 470. Idaho Agricultural Experiment Station.

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Table 1. Reactions of 18 bean varieties to the type and two variant strains of bean virus 1.

Variety	Reaction[a] to bean virus 1		
	Type strain	1943 strain[b]	1954 strain
Red Mexican, common	S	S	S
Burtner	S	S	S
Red Kidney	S	S	S
Pinto, UI-72	R	S	S
Pinto, UI-78	R	S	S
Pinto, UI-111	R	S	S
Red Mexican, UI-3	R	S	S
Red Mexican, UI-34	R	S	S
Great Northern, UI-16	R	R	S
Great Northern, UI-31	R	R	S
Great Northern, UI-59	R	R	S
Great Northern, UI-81	R	R	S
Great Northern, UI-123	R	R	S
Idaho Refugee	R	R	R
Idaho Bountiful	R	R	R
Puregold Wax	R	S	S
Improved Tendergreen	S	R	S
Topcrop	R	R	R

[a] R, resistant and S, susceptible.

[b] Variously referred to as Strain A, New York strain, New York 15 strain, and Burkholder's strain.

The principal distinction between the common bean-mosaic virus and the yellow bean-mosaic virus is that the common bean-mosaic virus is seed-transmitted and the yellow bean-mosaic virus is not. Inasmuch as it was not possible to visually distinguish the symptoms incited by the various strains of common bean-mosaic virus from those incited by yellow bean-mosaic virus in certain bean varieties, only a seed-borne virus was used for infectivity tests. Mosaic-free GN UI-123 seedlings were inoculated with juice extracted from a single seed-infected plant of the same variety. The inoculated seedlings were then utilized as the inoculum source for subsequent investigations of varietal reaction to the virus. The resistance of Great Northern UI-123 to the type strain and the variant strain of common bean-mosaic virus reduced the possibility of a mixture of virus strains. Use of a seed-borne virus likewise minimized the possibility of contamination with yellow bean-mosaic virus.

RESULTS

The initial attempt to transfer the apparently new virus from diseased GN UI-123 plants growing in the field to greenhouse-grown GN UI-123 seedlings met with only mediocre success. Transmission from 6 of 10 field plants which showed distinct symptoms of mosaic was accomplished. Of 42 seedlings inoculated from virus-infected field plants, only 9 developed symptoms characteristic of common bean mosaic. Subsequent transfers of the virus from these mechanically inoculated plants to additional seedlings of GN UI-123 were readily accomplished. Frequently, 100 percent of inoculated GN UI-123 seedlings developed mosaic symptoms.

Both common bean-mosaic and yellow bean-mosaic symptoms developed in GN UI-123 seedlings inoculated with crude plant extract from two of the six field-infected plants from which transmission was successfully accomplished. Certain of these inoculated seedlings developed symptoms more characteristic of yellow bean mosaic than of common bean mosaic. Symptoms on other seedlings appeared to be more characteristic of common bean mosaic than of yellow bean mosaic. Still others developed symptoms characteristic of both diseases.

Idaho Bountiful, Idaho Refugee, and Great Northern UI-123 seedlings were inoculated with crude plant extract obtained from the GN UI-123 plants first inoculated in the greenhouse. Again, Great Northern UI-123 seedlings developed symptoms characteristic of both diseases, but symptoms on Idaho Bountiful and Idaho Refugee were characteristic of yellow bean mosaic.

The plants of these three varieties were grown to maturity and the seed harvested. Plants of Idaho Bountiful and Idaho Refugee grown from this seed developed no symptoms of mosaic, but plants grown from the GN UI-123 seed developed classical symptoms of common bean mosaic only.

The seed-transmitted virus in GN UI-123 could not, in numerous attempts, be transmitted to either Idaho Bountiful or Idaho Refugee. It was assumed, therefore, that the two field plants in question carried both the new strain of common bean-mosaic virus and the yellow bean-mosaic virus.

Symptoms incited by the type strain of common bean-mosaic virus in susceptible bean varieties were indistinguishable from those of the new virus on GN UI-123. Symptoms varied from an indistinct granular mottle of the leaves to severe malformation. The infected plants were dwarfed, branched profusely, and set few pods. Leaves on such plants cupped downward, curled and twisted in various degrees. The mosaic pattern either appeared as dark bands along the major veins, with a clearing along the margin and between the veins of the leaf, or it appeared as dark green islands in a generally chlorotic leaf. The mosaic pattern was also characterized by an uneven growth rate in the leaf so that the darker areas were raised and gave the leaf a warty appearance. Plants of GN UI-123 grown from mosaic-infected seed frequently exhibited a distinct mottle of the primary leaves.

Varietal susceptibility to the virus recovered from GN UI-123 was determined in comparison with both the type strain of common bean mosaic and the variant strain reported in 1943. Bean varieties tested which carried the recessive factor for resistance to common bean mosaic (type strain) were all found to be susceptible to the virus recovered from GN UI-123 (Table 1). Varieties deriving common bean-mosaic resistance from Corbett Refugee (dominant factor for resistance to the type strain) were variable in reaction to the virus recovered from GN-123.

Common bean-mosaic-like symptoms were readily distinguished on all University of Idaho-introduced Great Northern varieties after being inoculated with the virus recovered from GN UI-123. Common Red Mexican and Burtner likewise were severely affected by the virus. Pinto and Red Mexican varieties originated by the University of Idaho were only moderately affected, and subinoculations to GN UI-123 were frequently necessary to confirm the presence of the virus. The garden bean varieties Idaho Refugee, Idaho Bountiful, and Topcrop did not develop symptoms of mosaic when inoculated with the seed-borne virus from GN UI-123. Subinoculations from these varieties were also negative. Plants of Improved Tendergreen and Puregold Wax developed characteristic symptoms of common bean mosaic when inoculated with virus recovered from GN UI-123. These last two varieties, although presumably carrying the dominant resistance derived from Corbett Refugee, also reacted in an unusual manner to the type and a variant strain of common bean-mosaic virus (3).

Visual comparison of the symptoms of the type strain, a variant strain, and the strain of common bean mosaic from GN UI-123 among inoculated common Red Mexican plants revealed that the virus from GN UI-123 caused the most pronounced growth depression and mottling of leaves, and the type strain had the least effect. Nonetheless, symptoms of all three strains on any given susceptible bean variety were so similar that they could not accurately be distinguished.

None of approximately 200 plants grown from seed harvested from the mosaic-infected GN UI-123 plants found in the field developed symptoms of mosaic.

Seed harvested from inoculated plants of GN UI-123 which developed characteristic symptoms of mosaic, however, consistently transmitted the virus to a small percentage of the resulting seedlings. The virus is also transmitted through seed of common Red Mexican.

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FUNGI ASSOCIATED WITH RED AND WHITE CLOVERS IN NEW HAMPSHIRE[1]

R. A. Kilpatrick[2]

Summary

Fungi associated with aerial and underground parts of red and white clovers were isolated and identified during a 3 1/2-year period. Twenty-two fungi were associated with red clover, while 39 fungi were identified from white clover. Four fungi, Colletotrichum trifolii, Leptodiscus terrestris, Sclerotium bataticola and Uromyces nerviphilus, are reported for the first time from New Hampshire and from New England.

Many fungi attack red and white clovers in the United States (2). In a previous paper (11), it was reported that several diseases of red and white clovers occur in New Hampshire.

Since 1955, diseased plants of red and white clovers have been examined. The objectives were 1) to determine the fungi associated with aerial and underground plant parts of red and white clovers; 2) to gain a better knowledge of disease symptoms; and 3) to learn more of the cultural characteristics and life history of pathogenic fungi attacking red and white clovers.

Diseased tissues were dipped momentarily in 50 percent ethyl alcohol, sterilized in 5 percent sodium hypochlorite (Clorox) for 2 minutes, and then transferred to Petri dishes containing Difco potato-dextrose agar. Five to 7 days later fungi developing from plated tissues were identified by microscopic examination. Another method of isolation consisted of placing non-sterilized, tissue-sections in a Petri dish moist chamber. Within 48 to 96 hours abundant sporulation occurred on the tissue fragments and the fungi were identified. In both cases, dishes were incubated at room temperature (68° to 90° F).

Twenty-two fungi were identified from red clover plants (Table 1). Of these, 16 have been reported as pathogenic: Cercospora zebrina Pass. (8), Colletotrichum destructivum O'Gara (16), C. trifolii Bain & Essary (15), Curvularia trifolii (Kauff.) Boed. (13), Cymadothea trifolii (Pers. ex Fr.) Wolf (8), Erysiphe polygoni DC. (8), Fusarium oxysporum Schlecht. (12), Kabatiella caulivora (Kirchn.) Karak. (8), Pseudopeziza trifolii (Biv.-Bern.) Fckl. (9), Pseudoplea trifolii (Rostr.) Petr. (8), Rhizoctonia solani Kuehn (12), Rhodotorula glutinis (Fres.) Harrison var. rubescens (Saito) Lodder (10), Sclerotinia trifoliorum Eriks. (17), Stagonospora meliloti (Lasch.) Petr. (8), Stemphylium sarcinaeforme (Cav.) Wiltshire (8), and Uromyces trifolii (Hedw. f.) Lév. var. fallens (Desm.) Arth. (1).

More detailed investigations were conducted on white clovers (Table 1). Of the 39 identified fungi, the following were most frequently isolated: Alternaria tenuis Nees ex Wallr., Cladosporium sp., Fusarium oxysporum, Mucor spp., Penicillium spp., and Rhizopus nigricans Ehr. Pathogenic fungi included Cercospora zebrina (8), Curvularia trifolii (14), Cymadothea trifolii (8), Erysiphe polygoni (8), Fusarium oxysporum (3), Leptodiscus terrestris Gerdemann (5), Pseudopeziza trifolii (9), Pseudoplea trifolii (8), Pythium sp. (7), Rhizoctonia solani (4), Rhodotorula glutinis var. rubescens (10), Sclerotinia trifoliorum (3), Sclerotium bataticola Taub. (4), Stagonospora meliloti (8), Stemphylium trifolii Graham (6), Uromyces nerviphilus (Grog.) Hotson (1), and U. trifolii var. trifolii-repentis (Liro) Arth. (1).

The pathogenicity of those fungi associated with roots and stolons, listed in Table 1, was checked by pure-culture, test-tube inoculations (12). The results on both red and white clover seedlings were in agreement with those previously reported (12).

The extent of economic losses in red and white clovers due to separate or simultaneous attacks by pathogenic fungi is still comparatively obscure. Severity of infection varies according to seasonal conditions, geographic location, age of plants, harvesting, management, insects, varieties, soil conditions, and possibly other factors. Studies are underway to deter-

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[1] Cooperative investigations of the Crops Research Division, Agricultural Research Service, United States Department of Agriculture and Departments of Botany and Agronomy, New Hampshire Agricultural Experiment Station, Durham. Published with the approval of the Director of the New Hampshire Experiment Station as Scientific Contribution No. 243.

[2] Plant Pathologist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture.

Table 1. Fungi isolated and identified from aerial and underground tissues of red and white clovers in New Hampshire[a].

Fungus	Leaves	Petioles	Roots	Seeds	Stems	Stolons
Absidia sp.			W			
Alternaria tenuis	R W	W	W	R W	R	W
Aspergillus flavus Lk. ex Fr.			W			
Aspergillus terreus Thom			W			
Cercospora zebrina	R W	W			R	
Chaetomium sp.	R W		W	R W		
Cladosporium sp.	R W	W	W	R W	R	W
Colletotrichum destructivum	W	W	R W			W
Colletotrichum trifolii			R			
Cryptococcus albidus (Saito) Skinner	W			W		
Curvularia trifolii	R W	W		W		
Cymadothea trifolii	R W					
Erysiphe polygoni	R W					
Fusarium oxysporum	W	W	R W	W		W
Fusarium roseum Lk.	W	W	W			W
Fusarium solani (Mart.) App. & Wr.			W			W
Gliocladium roseum (Link) Thom	W		W			W
Helminthosporium triseptatum ?				W		
Kabatiella caulivora	R				R	
Leptodiscus terrestris			W			
Mucor spp.	W	W	W	W		W
Neurospora sp.	W		W			
Nigrospora sp.			W			
Penicillium spp.	W	W	W	R W		W
Pestalotia sp.	R					
Phoma spp.	W		W	W		W
Pseudopeziza trifolii	R W					
Pseudoplea trifolii	R W	W		W		W
Pythium sp.			W			
Rhizoctonia solani	W		R W	W	R	W
Rhizopus nigricans	W	W	W	R W		W
Rhodotorula glutinis var. rubescens	R W			R W		
Rhodotorula mucilaginosa, (Jörgensen) Harrison	W					
Sclerotinia trifoliorum		W	R W		R	W
Sclerotium bataticola			W			
Stagonospora meliloti	R W					
Stemphylium sarcinaeforme	R					
Stemphylium trifolii	W	W		W		W
Thielaviopsis sp.			W			
Trichoderma lignorum (Tode) Harz			W			
Uromyces nerviphilus	W	W				
Uromyces trifolii fallens	R				R	
Uromyces trifolii trifolii-repentis	W	W				
Unidentified spp.	W	W	W	W		W

[a] R = red clover; W = white clover

mine the interrelationship of fungi and cold hardiness under controlled conditions.

The following fungi are reported for the first time from New Hampshire and from New England: Colletotrichum trifolii, Leptodiscus terrestris, Sclerotium bataticola, and Uromyces nerviphilus.

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RELATIONSHIP BETWEEN ROOT FEEDING INSECTS AND INCIDENCE OF CROWN AND ROOT ROT IN RED CLOVER

J. H. Graham and R. C. Newton[1]

Abstract

The results of two experiments conducted in the greenhouse indicated a relationship between injury and the incidence of crown and root-rot of red clover. Most rot developed in association with clover root borer injury, less with Sitona hispidula and very little in the controls. Relatively high incidence of rot occurred when crowns and roots growing in Fusarium infested soil were injured mechanically while almost no rot developed in steamed soil.

A reduction in vigor was observed in plants infested with the two insects.

An internal breakdown condition of unknown origin was observed in the crown tissue of red clover in all treatments.

Stands of red clover in northeastern United States usually are seriously depleted during the second year of growth. Reasons for the lack of persistence of this perennial are not known, although an insect-injury-disease complex is believed to be involved.

Two experiments were conducted in the greenhouse during the winters 1957-1958 and 1958-1959 to determine the effects of the clover root curculio, Sitona hispidula (F.), the clover root borer, Hylastinus obscurus (Marsh.), soil fungi, primarily Fusarium roseum (Lk.) Snyd. & Hans., and mechanical injury, separately and in combination on growth and incidence of crown and root-rot of Pennscott red clover.

MATERIALS AND METHODS

One-gallon glazed crocks were filled with greenhouse potting-soil and divided into three series of treatments. Two of the series were steamed for 8 hours at approximately 5 pounds' pressure. The fungicides captan and zineb were applied to one series of the steamed soil 10 days and again 20 days after the initiation of the experiment. Isolates of Fusarium spp. were cultured on "V8-juice" agar, the conidia and mycelium scraped off, and applied to the soil in the second series of treatments at the same intervals that the fungicides were added. Two holes were made with a test tube near the crown of each plant and filled with the inoculum. The third series of treatments consisted of non-steamed soil. Single seedlings of red clover, about 3 weeks old, were transplanted to the crocks and the following sub-treatments were initiated when the seedlings were 10 weeks old: 1) Eight adult Sitona collected in late fall in fields of legumes were added to the plants in each crock. 2) Six adult Hylastinus, obtained from second year red clover roots, were placed near the plant crowns of another group. 3) Eight Sitona and six Hylastinus were confined together as a third sub-treatment. Screen cages were placed over all plants infested with insects and removed after 7 weeks. 4) Plants of one sub-treatment were injured mechanically with a scalpel by making a small slit in the crown and cutting a few secondary roots. 5) A control consisted of plants receiving no injury or chemical treatment. Each treatment was replicated four times. Plants were allowed to wilt between waterings and were cut at full-bloom. Greenhouse temperatures were held between 20° and 25° C and daylength was increased to 13 hours with supplemental light. The experiment was terminated after 18 weeks.

The above test was repeated the following winter with these changes: 1) The non-steamed soil treatment was eliminated. 2) Fusarium roseum only was mixed with the soil. The isolates were moderately pathogenic to 2-month-old plants of red clover. 3) Twenty Sitona, instead of eight, were placed on each plant. 4) All plants in the experiment were caged separately. 5) The experiment was terminated after 13 weeks. 6) No fungicides were applied to the soil.

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Table 1. Green weight, in grams, aboveground parts[a] of red clover under various treatments in the greenhouse.

Sub-treatment	Test 1 (18 weeks)			Test 2 (13 weeks)	
	Steamed soil	Fusarium in steamed soil	Non-steamed soil	Steamed soil	Fusarium in steamed soil
Control	89	91	87	103	110
Mech. injury	76	85	70	93	115
Sitona	60	58	64	95	78
Borer	62	59	37	74	82
Sit. + Borer	53	51	55	75	76

[a]Average of three cuttings in 1957-1958; two in 1958-1959. Four replications per treatment.

Table 2. Development of root systems[a] of red clover grown under various treatments in the greenhouse.

Sub-treatment	Test 1			Test 2	
	Steamed soil	Fusarium in steamed soil	Non-steamed soil	Steamed soil	Fusarium in steamed soil
Control	8	6	7	9	8
Mech. injury	4	4	5	6	7
Sitona	4	4	7	7	7
Borer	5	6	3	6	6
Sit. + Borer	3	3	4	6	5

[a]Average of four replications based on index of 1-10 (10 most vigorous).

Table 3. Incidence of crown and root rot[a] of red clover grown under various treatments in the greenhouse.

Sub-treatment	Test 1			Test 2	
	Steamed soil	Fusarium in steamed soil	Non-steamed soil	Steamed soil	Fusarium in steamed soil
Control	0	2	13	<1	0
Mech. injury	0	31	40	1	5
Sitona	14	4	9	0	2
Borer	17	30	50	6	4
Sit. + Borer	31	43	33	5	5

[a]Average of four replications, based on percentage of root and crown area showing rot or discoloration.

Plants were periodically observed for vigor and green top-weights, and when the tests were ended the roots were examined for insect and mechanical injury and presence of root and crown rot. Isolations were made from the diseased plant tissues.

RESULTS

Seven of the 24 plants infested with borers were seriously damaged or completely cut by the adults in the first experiment within a few weeks and had to be replaced. In the second experiment two plants in the Sitona-root borer-Fusarium sub-treatment were killed during the first month, apparently by the interaction of the adult borers and Fusarium, and were replaced.

There were no consistent differences in the green weights of aboveground parts of plants (Table 1) among the three soil treatments; however, a reduction in both experiments was evident in treatments where insects were feeding on the roots. The differences in weights between the two experiments were due to the small amount of growth at the third harvest when the

experiment was terminated (1 month after second harvest). While the data reflect root damage, there was some leaf feeding during the time Sitona were caged on the plants.

At the conclusion of the experiments the root systems were rated for development (Table 2) and crowns and roots were rated for discoloration and rot (Table 3). The results indicate that root development was reduced by both mechanical and insect injury in most soil treatments.

In examining for prevalence of crown and root rot, lesions caused by Sitona larvae were found on roots of all plants to which the insects were added. Borer tunnels were evident in the crown and roots of all plants except five in the first experiment. These five plants were not considered in the borer-root ratings.

The amount of crown and root rot in the second experiment was much less than in the 1957-1958 experiment, probably because the second was run for a shorter period (13 weeks vs. 18 weeks). In addition, Sitona larvae for some unknown reason were either less active or active for a shorter period in the latter case.

Sitona larvae stripped some of the secondary rootlets and fed on the tap roots on all plants; however, the incidence of rot associated with the chewed areas was relatively low in all treatments.

Most crown and root rot was associated with borer damage. In the first test rot was moderate in steamed soil and severe in steamed soil with Fusarium and in non-steamed soil. Also, the incidence of rot was higher in association with both insects than with either separately. The addition of Fusarium to the soil increased the amount of rot associated with insect damage in the first test but not in the second. However, in both experiments a definite relationship was indicated between injury (mechanical and insect) and the incidence of root rot.

Isolations made from discolored crown and root tissue revealed mostly Fusarium spp. Rhizoctonia solani Kuehn was recovered in a few instances.

In the examination of crowns and roots for rot, an internal breakdown condition was discovered in the crown tissue near the crown-root zone of differentiation. The area developed in the pith and in early stages did not extend to the outer epidermis. It was present in 20 percent of the plants in all treatments in the two experiments and in isolations was not associated with any specific organism. The relationship of internal breakdown and longevity of red clover will be discussed in a later publication.

DISCUSSION

Results indicate that there is a relationship between injury and crown and root rot in red clover. A difference was observed between mechanical and insect injury. The former is instantaneous and discontinuous while the latter is continuous. It is believed that in steamed soil mechanical injuries healed before there was a buildup of Fusarium (from outside sources), thereby accounting for the absence or low incidence of rot. In contrast, the continuous feeding by insects provided a ready means of entry for the wound-requiring fungi. Fusarium spp. were isolated from the discolored areas on roots and crowns of plants in steamed, Fusarium-treated, and non-steamed soil series. The above explanation also would account in part for the greater reduction in vigor of plants infested with insects than in those injured mechanically.

Both greenhouse experiments were run for too short a time to observe the full impact of crown and root rots on longevity of red clover. The important fact is that the organisms became established in the plants. If the tests had been conducted for a longer period under conditions less favorable for the host than the pathogen (for example, drought and high temperature), as occurs in nature, the plants with the insect injury-rot complex probably would have been killed more readily than would the non-injured plants.

Although an insect-root rot relationship has been suggested in red clover, this is the first experimental verification of the phenomenon. It is not assumed that these are the only factors involved in the lack of persistence of red clover. The internal breakdown condition might be one factor. In addition, other pathogens (viruses, for example), as well as cultural and environmental conditions, are known to limit the life of red clover and other legumes.

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AN APPARATUS FOR THE STUDY OF THE MUTUAL EFFECTS OF ROOT EXUDATES ON PLANTS[1, 2]

Roger G. Lambert[3]

Summary

An apparatus has been designed which automatically mixes root solutions from two plants growing in sand culture. Preliminary experiments show that oats grown in association with alfalfa reduces the growth of alfalfa and alfalfa increases slightly the growth of oats.

INTRODUCTION

Historically, the study of the effect of one plant on another, as reviewed by Bonner (2), begins with observations by de Candolle in 1832. In 1903, Pickering (11) showed that the leachings from grass roots were deleterious to apple seedlings. Since that time, numerous reports of the production by one plant of substances which are inhibitory to itself or to other plants have appeared (1, 3, 8, 10, 12, 15). Other experiments (4, 5, 6, 7, 13, 14) indicate that the inhibiting effect of one plant on another may be explained by C/N ratios or by changes in the microflora of the soil. In 1937, Loehwing (9) pointed out the need for a method of studying root excretions to obtain more knowledge of root interactions.

The apparatus described herein was designed to intermix the root exudates from two plants growing in separate containers containing quartzite with a modified Hoagland's solution.

DESCRIPTION OF APPARATUS

A 40 x 40-inch tabletop attached to a 1 1/2-inch hydraulic cylinder powered by a hydraulic pump and a 1/4 h.p. electric motor moves up and down automatically through a 14-inch stroke (Fig. 1). The stroke is reversed by a four-way valve. When the tabletop reaches the top of the stroke, a lever switches the valve so that the oil is directed to the top of the cylinder and when the tabletop reaches the bottom of the stroke, the lever switches the valve so that the oil is redirected to the bottom of the cylinder. One complete cycle can be made in 90 seconds.

One-quart Styrene containers for growing the plants are placed on this movable tabletop. Each container has a hole bored in the side fitted with a rubber stopper through which a piece of glass tubing with a 90° angle bend is inserted (Fig. 2). The bent tube serves as a gauge in determining the solution level in the container and for removal of solution for periodic testing for minerals and organic materials. The hole in the bottom of the container is fitted with rubber tubing through which a short piece of glass tubing is inserted (Fig. 2). Pieces of nylon screen are placed over the holes, coarse gravel added, and the container filled with washed 8-mesh quartzite. This container is connected by blackened tygon tubing to another identical container on a stationary table. The stationary table is 7 inches above the movable tabletop when at the bottom of the stroke. Seeds are planted in the sand and a modified Hoagland's solution added to just saturate the sand in the container on the movable table when the table is at the bottom of the stroke.

As the movable tabletop rises, the solution bathing the roots in the first container flows through the tubing into the second container on the stationary table, carrying with it root exudate from the plants in the first container. As the tabletop completes the stroke and moves down, the solution flows back into the first container, carrying with it the added root exudate from the plants in the second container. By adjustment of the stroke length and the rate of oscillation, the level of the solution may be controlled so that the amount of moisture available to the plants in both containers is not a variable. Also by adjusting the rate of oscillation, the number of solution changes per unit time may be varied. At the rate of 90 seconds per stroke, 960 solution changes are made every 24 hours. Since the plants are in two separate containers,

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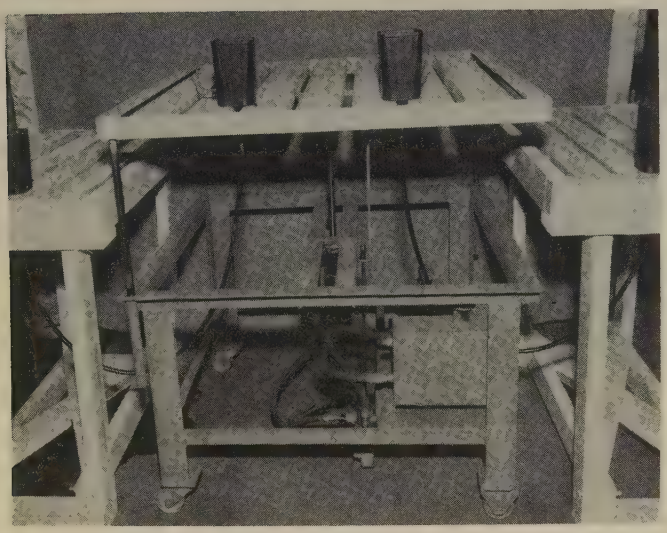

FIGURE 1. View of hydraulic table and two stationary tables with plant containers and associated tygon tubing.

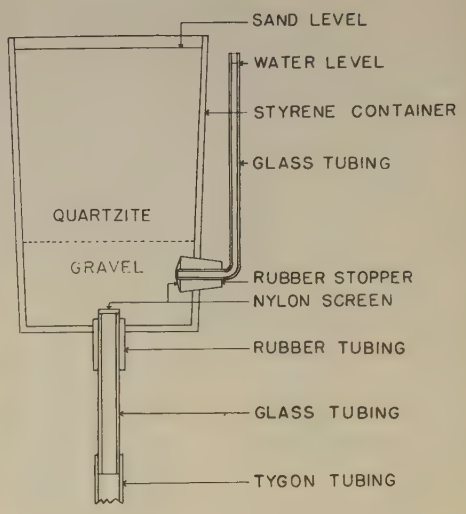

FIGURE 2. Cross section of Styrene container used to grow plants showing level gauge, tubing connections, and planting medium.

shading can be discounted as an explanation for a deleterious effect of one plant on another. The exchange of solution between containers permits aeration of the roots; thus this, too, is not a variable. Samples of the solution may be drawn off at intervals for other tests without disturbing the plants. With an apparatus of this size, 80 associations may be studied at one time. By inserting a fritted glass filter in the tubing between the two containers to prevent passage of soil particles or organisms, the effect of soil extracts, plant residues, and microorganism toxins on plant growth may be studied.

THE ASSOCIATION OF OATS AND ALFALFA

The following experiment was made using a similar apparatus to determine the effect of growing oats and alfalfa alone or in combination. Nine-cm funnels with large stems were fitted with a perforated plate and filled with quartzite. Four funnels on a stationary rack were connected to four funnels on a movable rack by tygon tubing. A 1/8 h.p. motor attached to a reducing gear raised and lowered the movable rack by means of a series of eccentric levers.

Fifteen seeds of Vernal alfalfa or Ajax oats were planted in each funnel and thinned to ten after 1 week. The funnels were connected in the following combinations:

Stationary	Movable
Alfalfa	Alfalfa
Alfalfa	Oats
Oats	Alfalfa
Oats	Oats

A modified Hoagland's solution was added initially and fresh solution added weekly thereafter. Water lost through evapotranspiration was replaced with distilled water. The movable rack was raised 7 inches above and lowered 7 inches below the stationary rack at the rate of two cycles per minute for 5 hours each day. When the apparatus was not operating the racks were kept at the same level.

The average heights of the plants after growing 4 weeks under these conditions are given in Table 1.

From these preliminary data the association of alfalfa and oats appears to be detrimental to alfalfa and slightly beneficial to oats under these conditions during the early stages of growth.

The study of this association and other plant associations is being continued, using the new hydraulic root exudate exchanger. This apparatus will facilitate the study of the interaction of inorganic ions and of organic compounds including possible growth-regulating compounds

Table 1. The effect on height of growing alfalfa and oats in nutrient sand culture in different combinations in which the root solution from one plant is intermixed with that from the other plant.

Stationary[a]	Average height[b] (in cm)	Movable[a]	Average height[b] (in cm)
Alfalfa	11.1	Alfalfa	9.4
Alfalfa	6.9	Oats	24.5
Oats	24.6	Alfalfa	5.1
Oats	22.0	Oats	20.6

[a]Stationary and Movable indicate whether the plants were supported by the stationary or movable funnel rack.
[b]Averages are based on two experiments replicated in time with 10 plants in each funnel.

produced by plants growing in association with other plants.

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SYMPTOMS INDICATING XYLOPOROSIS[1] IN UNINOCULATED ORLANDO TANGELO SEEDLINGS

G. G. Norman, R. R. Nixon, Jr., L. Horne,[2] and J. T. Grantham[3]

Seedlings of Orlando Tangelo (Citrus reticulata x C. paradisi) have been used as the indicator plants for xyloporosis virus (1, 2, 3) in the Florida Citrus Budwood Registration Program since it was started in 1953.

The Orlando test plants were obtained from a commercial nursery (Grand Island Nurseries, Eustis, Florida) from outdoor seed beds planted in 1952 and 1953. The identity of the trees producing the seed was not recorded, hence their virus content could not be determined. The seedlings were systematically rogued for variant types and when approximately 4 feet high were transferred without soil to the test plot site in Winter Haven. After transplanting, the central trunk of each seedling was lopped off 20 to 24 inches above ground level. Periodic examination of some seedlings for phloem discoloration, wood pitting, or other indications of the presence of xyloporosis was negative until the Spring of 1959. To date nine uninoculated Orlando Tangelos show typical xyloporosis symptoms[4] (Figs. 1-4). The roots of two apparently xyloporosis-infected but uninoculated control trees planted 4 feet from infected test trees

FIGURE 1. Uninoculated Orlando seedling, showing chlorotic foliage, small leaves, upper limbs dead or dying back. Adjacent trees are healthy Orlando and Sweet Orange seedlings.

FIGURE 2. Uninoculated Orlando seedling used as a control, about 8 years from seed. No root grafts were found on any of the infected seedlings.

were exposed by the use of water pressure to remove all soil but no evidence of root grafts could be found. Seven uninoculated seedlings, apparently xyloporosis-infected, are located in areas of the nursery where they are surrounded by other seedlings with no known source of infection within 100 feet of the affected trees.

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[1]These trees have been indexed to other seedlings to confirm transmissibility of the causal agent.
[2]Inspectors, Citrus Budwood Registration Program, State Plant Board of Florida.
[3]Nurseryman, Citrus Budwood Registration Program, State Plant Board of Florida.
[4]These trees have been examined by J. F. L. Childs, who concurs in symptom diagnosis.

FIGURE 3. Close-up of Figure 2, showing typical xyloporosis wood pitting, discoloration of wood and cambial face, and phloem deterioration.

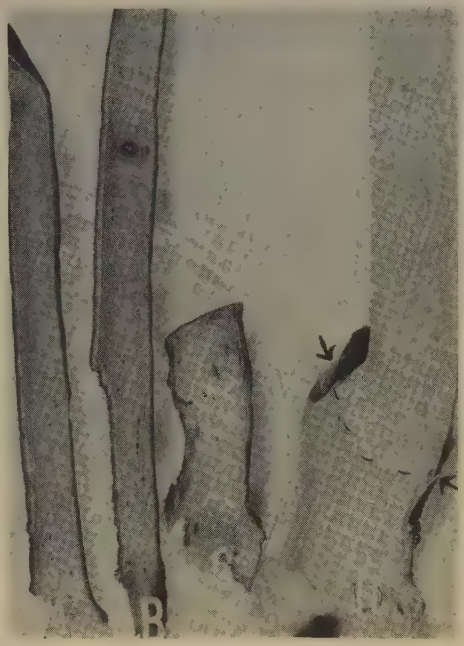

FIGURE 4. A, B, and C are bark strips from an infected seedling. The cambial face shows typical xyloporosis symptoms. D is the bare trunk. Dotted line shows where trunk was lopped after transplanting.

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STATE PLANT BOARD OF FLORIDA, WINTER HAVEN

AN INVESTIGATION OF THE PRESENCE OF A MORPHOLOGIC INDICATOR OF LOOSE-SMUT INFECTED BARLEY SEEDLINGS[1]

A. H. Andersen and Steve Lund[2]

Summary

Twenty barley varieties were tested for morphological seedling characteristic indicating loose smut infection. Only one variety, Weibull's 5425, consistently produced shortened leaf-sheaths on seedlings of infected plants.

INTRODUCTION

The systemic nature of loose smut infection, caused by Ustilago nuda (Jens.) Rostr., prevents the investigator from determining the amount of infection until the barley heads emerge from the boots. In winter barley, unless the plants are vernalized, this means a full year is required for determination of infection. Ohms and Bever[3] found that a shortened leaf-sheath indicated loose smut infection in two varieties of winter wheat, but they observed a characteristic difference between the two varieties.

Since such a marker would speed up the breeding for resistance, a study was initiated in 1955 on Calhoun winter barley. Later, spring barley varieties were included in the study.

RESULTS AND DISCUSSION

A lot of winter barley of the variety Calhoun, with a known infection of 13 percent, was obtained from R. E. Earhart, Clemson College, Clemson, South Carolina. In preliminary studies, the length of coleoptiles, leaves, and leaf-sheaths was measured. The only consistent pattern was found to be either a shortened third or a fourth leaf-sheath on some of the plants similar to that described by Ohms and Bever[3]. In further studies with Calhoun, it was found that about 13 percent of the seedlings possessed a shortened leaf-sheath, as shown in Figure 1. This indicated that a marker of loose-smut infected seedling might also occur in winter barley.

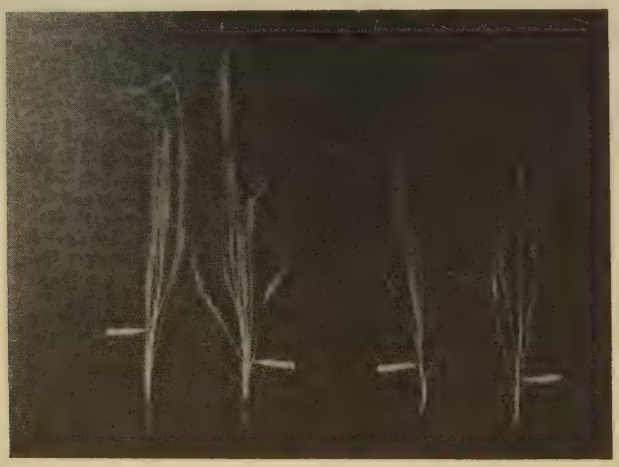

FIGURE 1. Three-week-old seedlings of the variety Calhoun showing shortening of leaf-sheath. From left to right, plants show normally elongated third leaf-sheath, shortened third leaf-sheath, normally elongated second leaf-sheath, and shortened second leaf-sheath.

Of the 625 seeds of the Calhoun variety which were planted in the greenhouse in January 1956, 425 seedlings emerged. The plants were exposed to artificial light from five 200 Watt bulbs from sunset to 11:00 p.m. to hasten the heading. The seedlings were examined 14 days after emergence, and 10.6 percent possessed the shortened leaf-sheath. All plants were kept in the greenhouse until June, but only a few plants produced heads. Nearly all the headed plants were smutted, but none of the smutted plants possessed the shortened leaf-sheath. Since

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[3] Ohms, R. E., and W. M. Bever. 1955. Type of seedling reaction of Kawale and Wabash winter wheat to three physiologic races of Ustilago tritici. Phytopathology 45: 513-516.

the heading was not uniform, there appeared to be no relationship between the shortened leaf-sheath and infection, although no definite statement to the effect could be made. In October 1956 three flats, 20 by 14 inches in size, were planted with 100 seeds in each flat. The seeds were planted at a depth of 3/4 of an inch. Three other flats of the same size were planted with 100 seeds each at a depth of 3 inches to discover the influence of planting depth on the appearance of the shortened leaf-sheath. The plants were exposed to artificial light from 11:00 p.m. to 2:00 a.m. When the seedlings were examined, the shortened leaf-sheath appeared as in the former experiment. Depth of planting had no influence on the appearance of this characteristic. After plants with the shortened leaf-sheath had been tagged, the flats were transferred outside the greenhouse for vernalization. In February the flats were returned to the greenhouse. At heading time, about 12 percent of the plants were smutted, but none of the smutted heads were on the tagged plants.

A study was also conducted in the greenhouse with 19 artificially inoculated varieties from the U.S.D.A. Uniform Spring Nursery. The seedlings were examined 3 weeks after emergence for the shortened leaf-sheaths that appeared in nearly all varieties. None of the marked plants produced smutted heads, except for one variety, Weibull's 5425. The measurements in inches between leaf blades of Weibull's 5425 plants are presented in Table 1.

Table 1. Measurements in inches between leaf blades of 13 3-week-old plants of the variety Weibull's 5425, which had been inoculated with loose smut, and response of mature plants.

Plant number	1-2	2-3	3-4	4-5	5-6	Mature plant response
1	1.00	1.00[a]	2.06	0.50	2.13	
2	0.75	0.13[a]	1.56	0.75	1.94	Smutted
3	1.00	0.00[a]	1.56	1.75		Smutted
4	1.38	0.19				Died
5	1.00	1.31	1.56	0.25	1.31	
6	1.13	1.19	2.06	0.80	3.13	
7	1.13	0.00[a]	1.63	1.19	2.00	Smutted
8	1.13	1.91	1.89	1.25	1.25	
9	1.13	1.13	1.06	0.50	3.19	
10	1.06	1.06	1.25	2.31	1.44	
11	0.75	1.19	1.63	0.56	2.00	
12	1.00	1.25	0.63	1.56	0.75	
13	0.00[a]	1.25	1.00	0.69	0.00[a]	Smutted

a Indicated presence of the marker.

Four of the 13 plants possessed the shortened leaf-sheath and only these four produced smutted heads. Three of the four possessed a shortened third leaf-sheath, but the fourth showed shortening of both the second and sixth leaf-sheath.

An attempt was made to determine the occurrence of the shortened leaf-sheath in the field on artificially inoculated spring barley. Field demonstrations were difficult because of early tillering of the seedlings, but the shortened leaf-sheaths appeared in several varieties, and these were tagged. None of the tagged plants produced smutted heads.

CONCLUSION

In the search for a seedling marker for infection of loose smut in barley, only one variety showed promising results out of 20 varieties tested. Ohms and Bever[3] found a marker in two winter wheat varieties, but they found a characteristic difference between the marker in the two varieties. This, together with the present results, indicates that the marker is a varietal characteristic. This limits the practical use of this as an indicator, since each variety must be tested for the presence or absence of it. Determination of this characteristic was made most easily in a greenhouse because tillering of field-grown plants made measurements difficult.

NEW JERSEY AGRICULTURAL EXPERIMENT STATION, NEW BRUNSWICK

AN ECOLOGICAL STUDY OF THE PATHOGENICITY OF DIPLODIA MAYDIS ISOLATES INCITING STALK ROT OF CORN[1]

H. C. Young, Jr.,[2] Roy D. Wilcoxson,[3] Marvin D. Whitehead,[4] J. E. DeVay,[5] C. O. Grogan,[6] M. S. Zuber[6]

Summary

Isolates of Diplodia maydis and certain corn single crosses were exchanged between Minnesota, Missouri, and Oklahoma. The corn entries were inoculated with each isolate in each of the three States during 1954, 1955, and 1957. In general, the isolates of the fungus were more pathogenic in States where they originated. All isolates were more pathogenic in Missouri and Oklahoma than in Minnesota. During 1955, when hot, dry weather prevailed in Minnesota, isolates from Missouri and Oklahoma were more pathogenic in Minnesota than was the isolate from Minnesota. The single crosses were generally more resistant in Minnesota than in Missouri and Oklahoma, but resistance varied with fungus isolates, single crosses, locations, year of the test, and with all of the interactions of the major factors studied.

INTRODUCTION

Prevalence and severity of stalk rot in corn are known to be influenced by a number of factors (1, 2, 3, 4, 5, 6, 7, 8, 9). The work reported here was undertaken to learn more about the operation of some of the major factors over a wide geographical and climatological area.

MATERIALS AND METHODS

Corn lines that differed in their susceptibility to stalk rot and isolates of Gibberella zeae (Schw.) Petch and Diplodia maydis (Berk.) Sacc. were exchanged between Minnesota and Missouri and between Minnesota and Oklahoma in 1953. The types of corn used in 1954, 1955, and 1957 were F_1 single crosses, hereafter referred to as entries. In 1954, 1955, and 1957, however, D. maydis and corn entries were sent from each station to the other two stations and studies with G. zeae were discontinued. The same isolates were used in 1955 and 1957 as were used in 1954. Because of drought, the tests in Oklahoma were successful in only 1953 and 1957.

Each corn entry was inoculated by inserting toothpicks bearing inoculum into the second internode above the ground 1 to 2 weeks after tasseling. In three replications at each station 10 plants of each entry were tested. Stalk rot notes were taken approximately 1 month after inoculation by splitting the stalks and rating the amount of rot on the following scale: 1) less than 25 percent of the inoculated internode rotted, 2) 25 to 50 percent of the internode rotted, 3) 50 to 75 percent of the internode rotted, and 4) more than 75 percent of the internode rotted. If internodes adjacent to the one inoculated also became rotted, the ratings were scored 5 or

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[1]Cooperative investigations, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, and the Minnesota, Missouri, and Oklahoma Agricultural Experiment Stations. Paper No. 4142, Scientific Journal Series, Minnesota Agricultural Experiment Station; Journal Article No. 2001, Missouri Agricultural Experiment Station; Journal Manuscript No. 487, Oklahoma Agricultural Experiment Station. The authors gratefully acknowledge the advice and assistance of Drs. J. J. Christensen, M. E. Michaelson, Thor Kommedahl, E. H. Rinke, Leon Wood, P. N. Nair, J. S. Brooks, and Mr. R. P. Covey.

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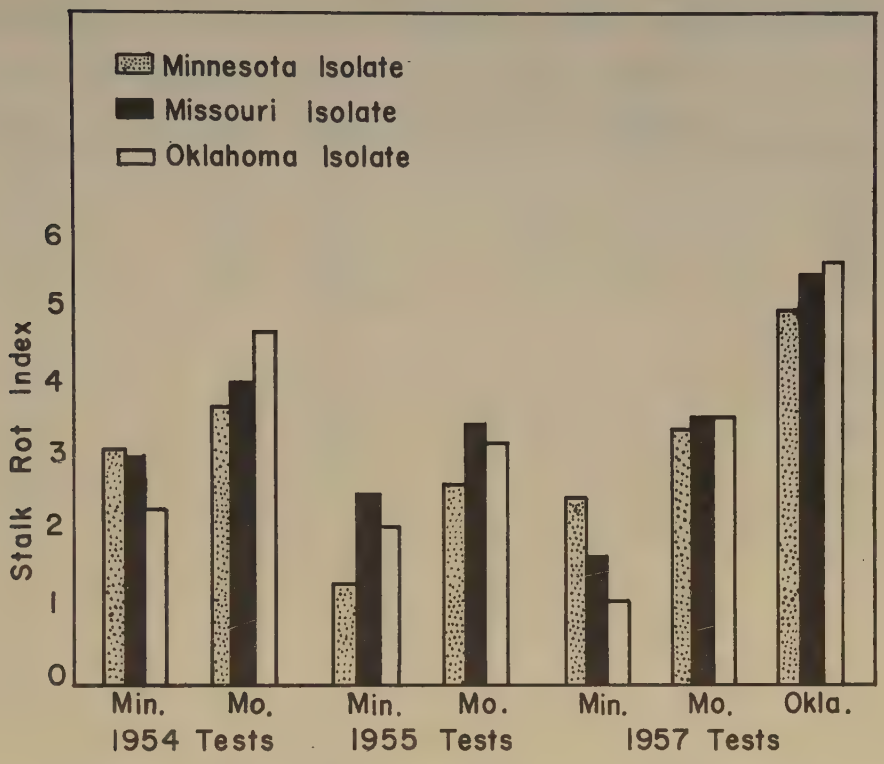

FIGURE 1. Stalk rot index for three isolates of Diplodia maydis tested during 1954, 1955, and 1957 in Minnesota, Missouri, and Oklahoma.

higher, the score depending on the extent of the rot.

The rate of radial spread of colonies of the isolates of D. maydis was studied by transferring 5-mm discs of agar bearing a uniform amount of mycelium to Petri dishes containing 20 ml of potato-dextrose agar. These cultures were held at 12°, 25°, 31°, and 36° C for 48, 72, 96, and 120 hours before colony diameters were measured.

RESULTS

The isolate of G. zeae from Minnesota was most virulent at each station in 1953. Amounts of stalk rot incited by the different isolates of D. maydis were similar in Minnesota, but in both Oklahoma and Missouri the isolates from those States caused more rot than did the Minnesota isolate.

The amount of stalk rot incited by the three isolates of D. maydis in the several tests made in 1954, 1955, and 1957 is shown in Figure 1. In 1954 and 1957 each isolate caused more rot in the area in which it was native. Thus, in Minnesota the Minnesota isolate caused more rot, while the isolates from Oklahoma and Missouri caused more rot at the southern stations. The Missouri isolate was intermediate in virulence to the other two isolates in the 1954 and 1957 tests; whereas in 1955 the Missouri isolate was most virulent. Statistical analysis of the data indicated that in Missouri in 1954 each isolate caused a significantly different amount of rot, but in Minnesota the Oklahoma isolate caused significantly less rot. In Minnesota in 1957 each isolate produced an amount of rot that was significantly different from that caused by the other two, but in Oklahoma and Missouri the Minnesota isolate caused significantly less rot than the others.

The tendencies noted for 1954 and 1957 did not apply in 1955. The isolates from Missouri and Oklahoma caused more stalk rot than did the isolate from Minnesota in the tests in both Minnesota and Missouri. The differences between southern isolates in the Missouri tests were not statistically significant. The Missouri isolate caused more rot than did the Oklahoma isolate, which, in turn, was more virulent than the Minnesota isolate in Minnesota.

All isolates in all tests caused more stalk rot in Oklahoma and Missouri than in Minnesota.

Table 1. Mean stalk rot reactions of corn F_1 single crosses inoculated with Minnesota, Missouri, and Oklahoma isolates of Diplodia maydis in Minnesota and Missouri in 1954.

Corn entries	Minnesota tests with isolates from			Missouri tests with isolates from			Mean for entries tested in	
	Minn.	Mo.	Okla.	Minn.	Mo.	Okla.	Minn.	Mo.
Minnesota corn entries:								
A73 x A334	2.8[a]	3.1	3.3	3.8	4.8	5.0	3.1	4.5
Ill 4226 x A334	2.3	2.8	2.4	3.6	4.9	5.4	2.5	4.6
Oh 51A x A334	2.6	3.1	2.6	3.3	3.9	4.7	2.8	4.0
A334 x A334	3.6	3.0	2.5	5.2	5.5	5.7	3.0	5.5
A340 x A334	2.5	3.3	1.8	2.9	4.0	4.8	2.5	3.9
Missouri corn entries:								
L317 x WF9	3.1	3.7	1.9	3.8	4.2	4.6	2.9	4.2
Mo 940 x T8	3.1	1.4	2.0	4.2	3.5	4.7	2.2	4.1
Mo 940 x WF9	2.5	1.3	2.2	4.3	4.3	4.9	2.0	4.5
Ky 27 x T8	3.2	1.4	2.5	3.2	2.6	4.4	2.4	3.4
Ky 27 x WF9	2.8	2.2	1.5	3.4	3.4	4.2	2.2	3.7
T8 x WF9	2.8	2.6	1.8	2.8	3.2	3.8	2.4	3.3
Mo 940 x L317	3.7	3.4	2.1	4.2	4.8	5.4	3.1	4.8
Ky 27 x 187-2	2.0	2.0	2.1	3.8	3.6	4.7	2.0	4.0
Ky 27 x CI 7	3.7	3.3	2.1	3.2	3.8	4.5	3.0	3.8
CI 7 x K4	3.9	3.3	1.6	4.3	4.5	5.1	2.9	4.6
Oklahoma corn entries:								
K4 x CI 21E	3.8	3.6	2.5	4.6	4.8	4.9	3.3	4.8
K4 x 119-1-6-2-2	3.8	3.5	3.4	3.3	4.3	4.9	3.6	4.2
K4 x 115-1-1-3-2	3.5	3.5	2.7	3.6	4.6	5.1	3.2	4.4
K4 x C17	3.8	3.7	2.4	4.3	4.2	5.0	3.3	4.5
K4 x 111-3-3-3	3.1	3.7	2.4	3.8	4.6	4.5	3.1	4.3
Ok 12 x CI 21E	3.5	3.5	2.7	4.0	3.1	4.7	3.2	3.9
K4 x Ok 12	3.5	3.7	2.4	3.9	3.4	5.0	3.2	4.1
K4 x 122-1-3-3-2	3.8	4.0	2.8	3.2	4.2	5.0	3.5	4.1
K4 x K201	3.5	3.7	2.9	3.5	3.8	4.6	3.4	4.0
Ky 5 x CI 7	4.1	4.3	2.5	4.8	4.8	5.2	3.6	4.9

[a]Average of 30 plants in three replications, LSD, 5% level for corn F_1 single crosses tested in Minnesota is 0.4 and in Missouri 0.9.

The stalk rot reactions of the several entries of corn tested in 1954, 1955, and 1957 are shown in Tables 1, 2, and 3, respectively. In each test some of the entries were more resistant to stalk rot than were others; but, in general, all entries had less stalk rot in Minnesota than in the other two States. During 1954 it appeared that corn entries from Missouri were more resistant than were those from the other two States; but, during 1955, the Missouri entries were the most susceptible in Minnesota tests and during 1957 the differences between corn entries from the three States were not apparent.

In 1954, K4 x C.I. 7 in Minnesota was most resistant to the Oklahoma isolate, but in Missouri it was most susceptible to the Oklahoma isolate. This single cross was most susceptible to the Oklahoma isolate in Minnesota in 1955, but it was only moderately susceptible in Missouri. In 1957 in Minnesota, K4 x C.I. 7 was most susceptible to the Oklahoma isolate. Similar behavior was noted for several other corn entries, which indicates that resistance to stalk rot may vary considerably with environmental conditions as well as with corn genotype and fungus isolates.

Because the Minnesota isolate of D. maydis was apparently most virulent during seasons of relatively greater rainfall and lower temperatures and the Oklahoma and Missouri isolates were most virulent under hot, dry conditions, laboratory studies were made to determine whether temperature requirements for radial spread of colonies on potato-dextrose agar were different for the three isolates of D. maydis. D. maydis from Minnesota grew best at 25° C and made better growth at 12° than did the other two isolates (Table 4). In contrast, the isolates from Missouri and Oklahoma grew best at temperatures above 25°, while the growth of the Minnesota isolate was negligible at these temperatures.

Table 2. Mean stalk rot reactions of corn F_1 single crosses inoculated with Minnesota, Missouri, and Oklahoma isolates of Diplodia maydis in Minnesota and Missouri in 1955.

Corn entries	Minnesota tests with isolates from			Missouri tests with isolates from			Mean for entries tested in	
	Minn.	Mo.	Okla.	Minn.	Mo.	Okla.	Minn.	Mo.
Minnesota corn entries:								
A334 x A73	1.1[a]	2.7	2.5	3.0	2.3	3.3	2.1	2.8
A334 x Ill 4226	1.3	3.0	2.0	3.0	3.7	3.3	2.1	3.3
A334 x Oh 51A	1.2	2.4	2.0	3.0	3.7	3.3	1.9	3.3
A334 x A340	1.6	2.1	2.3	3.3	3.7	4.0	2.0	3.7
A334 x A334	1.5	3.2	3.0	3.0	2.7	3.7	2.6	3.1
Missouri corn entries:								
Ky 27 x T8	1.2	2.2	2.2	2.3	3.2	2.8	1.8	2.8
Ky 27 x WF9	1.3	2.9	2.2	1.7	3.0	2.0	2.1	2.2
Mo 940 x L317	1.3	3.6	2.0	2.7	4.2	4.2	2.3	3.7
Mo 940 x T8	1.6	3.5	1.9	2.7	3.8	2.8	2.3	3.1
Mo 940 x WF9	1.3	3.3	2.3	2.7	3.7	3.5	2.2	3.3
L317 x WF9	1.2	2.9	1.5	2.0	3.8	3.2	1.8	3.0
Oklahoma corn entries:								
K4 x Ok 12	1.3	2.6	1.2	2.5	3.7	3.2	1.7	3.1
K4 x Ok 115-1	1.8	1.7	2.0	3.2	4.0	3.5	1.8	3.6
K4 x Ok 11	1.3	2.3	2.6	2.7	3.5	3.2	2.1	3.1
K4 x K201	1.0	1.3	2.8	3.3	4.0	3.8	1.7	3.7
K4 x CI 7	1.1	1.6	2.2	2.7	3.8	3.2	1.6	3.2

[a]Average of 30 plants in three replications; LSD, 5% level for corn F_1 single crosses tested in Minnesota is 0.2 and in Missouri 0.8.

DISCUSSION

Although only one isolate of each fungus from each State was used in this work, the isolates differed in pathogenicity and in their reaction to environment in the development of corn stalk rot caused by D. maydis. It is possible that a somewhat different picture of stalk rot could be obtained if other isolates were tested in still other localities and years. Preliminary work done in 1953 with G. zeae suggested that the amount of stalk rot caused by different isolates of this fungus might be different from that caused by D. maydis. More extensive work should be done, therefore, to determine whether the effects noted in this work have general application.

The present study demonstrates the necessity for working within the framework of an adequate experimental design when stalk rot is being investigated. Not only were the obvious variables (pathogen isolates, corn entries, locations, and years) statistically significant in their effect on stalk rot, but all the interactions were important also in altering the development of stalk rot.

The variable pathogenicity of the several fungus isolates studied during 1954, 1955, and 1957 appeared to be related to their temperature requirements as shown by radial spread of colonies. Thus, the Minnesota isolate was usually more pathogenic in Minnesota, where the growing season is commonly cool and moist; and it also grew best at a low temperature. The isolates from Missouri and Oklahoma generally were more pathogenic in those States and grew better at higher temperatures. The growing season in Minnesota in 1955 was unusually dry and hot, and during that season the isolates from Missouri and Oklahoma were more pathogenic in Minnesota than was the native isolate.

Corn entries generally were more resistant to stalk rot caused by D. maydis in Minnesota than in Missouri and Oklahoma. While this suggests that it might be easier to obtain stalk-rot-resistant hybrids for Minnesota, it should be remembered that in most years Fusarium species are more prevalent causes of stalk rot in Minnesota than is D. maydis, which is the most important cause of stalk rot in Missouri and Oklahoma. For this reason hybrids resistant to stalk rot in southern regions might not be resistant to stalk rot in Minnesota, and vice versa.

Table 3. Mean stalk rot reactions of corn F_1 single crosses inoculated with Minnesota, Missouri, and Oklahoma isolates of Diplodia maydis in Minnesota, Missouri, and Oklahoma in 1957.

Corn entries	Minnesota tests with isolates from			Missouri tests with isolates from			Oklahoma tests with isolates from			Means for entries tested in		
Source and Strains	Minn.	Mo.	Okla.	Minn.	Mo.	Okla.	Minn.	Mo.	Okla.	Minn.	Mo.	Okla.
Minnesota corn entries:												
A334 x Oh 51A	2.5[a]	1.9	1.0	3.1	3.8	3.6	5.4	5.4	6.2	1.8	3.5	5.6
A334 x A340	3.1	1.4	1.0	3.6	4.0	3.9	5.1	5.6	5.9	1.8	3.8	5.5
A334 x Ill 4226	2.0	1.2	1.0	3.1	3.7	3.6	5.0	6.0	5.7	1.4	3.5	5.6
A334 x A322	2.4	1.5	1.8	3.3	3.6	3.7	5.7	5.5	6.1	1.9	3.5	5.5
A334 x A73	2.6	1.6	1.1	3.5	3.9	3.7	5.7	6.0	6.8	1.8	3.7	6.2
Missouri corn entries:												
Mo 940 x WF9	2.3	1.5	1.0	3.6	4.0	3.9	4.3	5.0	4.1	1.6	3.8	4.5
T8 x WF9	2.1	1.3	1.0	2.9	3.6	3.2	4.0	3.9	3.9	1.5	3.2	3.9
Mo 940 x T8	2.9	1.6	1.1	3.1	3.6	3.4	4.3	5.1	4.8	1.8	3.4	4.7
T8 x L317	1.9	1.9	1.0	3.4	3.5	3.7	3.9	5.4	5.4	1.6	3.5	4.9
Mo 940 x L317	2.1	2.9	1.0	3.8	3.7	3.9	7.0	7.0	7.1	2.0	3.8	7.0
Oklahoma corn entries:												
K4 x CI 7	3.6	1.8	1.2	3.8	3.3	3.9	5.8	5.6	6.6	2.2	3.7	6.0
CI 7 x Ok 12	2.2	1.3	1.0	3.3	3.1	3.3	4.6	5.9	6.4	1.5	3.2	5.7
38-11 x Ok 15	2.8	2.8	1.1	3.4	3.4	3.5	4.5	4.9	5.6	2.2	3.4	5.0
K201 x L4	2.3	1.6	1.1	3.3	3.3	3.5	5.0	5.4	5.8	1.7	3.4	5.4
K201 x 38-11	2.9	2.7	1.0	3.6	3.3	3.9	5.8	5.6	5.6	2.2	3.6	7.3

[a]Average of 30 plants in three replications; LSD, 5% level for corn F_1 single crosses tested in Minnesota 0.5; in Missouri 0.1; and in Oklahoma 0.4.

Table 4. Average diameters of colonies of Diplodia maydis from Minnesota, Missouri, and Oklahoma growing at different temperatures for different periods of time.

Temperature (°C) and Isolate	Colony diameter (mm)[a] at indicated age (in hours)			
	48	72	96	120
12:				
Minnesota	0	0	9	10
Missouri	0	0	6	8
Oklahoma	0	0	6	8
25:				
Minnesota	60	90	90	90[b]
Missouri	60	88	90	90
Oklahoma	20	53	69	89
31:				
Minnesota	53	53	58	58
Missouri	47	64	71	71
Oklahoma	34	37	90	90
36:				
Minnesota	0	0	0	6
Missouri	6	6	9	9
Oklahoma	8	8	9	12

[a]Average of six plates.
[b]Colony covered the agar surface completely.

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THE VIRUS TOLERANCE OF FRAGARIA CHILOENSIS COMPARED WITH THE MARSHALL VARIETY[1]

P. W. Miller and G. F. Waldo[2]

One of the most effective ways to control any plant disease is to grow varieties that are resistant or tolerant.

During our strawberry virus investigations, we have had an opportunity to test the resistance of certain wild strawberry species to various viruses and combinations thereof. One of these, Fragaria chiloensis, was found to possess a high degree of tolerance to most strawberry virus components present in the Pacific Northwest. A discussion of the virus tolerance of 17 different clones of F. chiloensis collected at random along the West Coast from Del Norte, California to Nelscott, Oregon compared with the Marshall variety follows.

METHODS

Three mature potted plants of each clone of F. chiloensis were inoculated with known viruses, using Miller's modification of Bringhurst and Voth's excised leaf-petiole graft technique[3, 4]. The viruses were introduced singly and/or in combinations of two or more. Two leaf grafts of each component were made on each plant. The inoculated plants were then placed in a polyethylene film moist chamber for about 10 days, after which they were incubated in the greenhouse at an average temperature of 70° F. Untreated plants of the same clone were maintained as checks for comparison.

With respect to the Marshall plants inoculated, in some cases the subclone inoculated already contained the latent A virus as shown by previous indexing. In such cases, only other viruses were added. It should be stated that the Marshall was used as a control to measure the anticipated efficacy of the inoculations and to relate the probable symptoms.

FIGURE 1. An F. chiloensis plant infected with the Latent A, mild yellow edge and veinbanding strawberry virus components; from inoculations by excised leaf-petiole graft technique. Note absence of any visual leaf symptoms.

FIGURE 2. A Marshall plant infected with Latent A, mild yellow edge, mottle, and veinbanding strawberry virus components; from inoculations by excised leaf-petiole grafts. Note relative severity of virus symptoms.

[1]Cooperative investigations by Crops Research Division, Agricultural Research Service, United States Department of Agriculture and Oregon Experiment Station.

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[3]Bringhurst, R. S., and V. Voth. 1956. Strawberry virus transmission by grafting excised leaves. Plant Disease Reptr. 40: 596-599.

[4]Miller, P. W. 1958. Comparative efficiency of excised leaf-petiole grafts and stolon grafts for transmitting certain strawberry viruses. Plant Disease Reptr. 42: 1043-1047.

Table 1. The virus tolerance of Fragaria chiloensis compared with the Marshall variety, 1958-1959.

Plant	Clone numbers	Viruses introduced[a]	Symptoms[b]
F. chiloensis	1, 2, 8, 9, 13, 14, 17	Mild yellow edge	No leaf symptoms[c]
	10	Latent A + mild yellow edge	do.
	4	Latent A + veinbanding + mild yellow edge	do.
	12	Mottle + veinbanding + mild yellow edge	do.
	5, 16	Latent A + mottle + veinbanding + mild yellow edge	do.
	6	Latent A + veinbanding + mottle + crinkle + mild yellow edge	do.
	7	Latent A + Latent C + mottle + veinbanding + crinkle + mild yellow edge	do.
	3	Latent A + veinbanding	do.
	11, 15	Veinbanding + mottle	do.
Marshall	1	Latent A + veinbanding	Moderate leaf symptoms; plant slightly dwarfed
	2	Latent A + mild yellow edge + veinbanding	Severe leaf symptoms; plant greatly dwarfed
	3	Veinbanding + mottle	Severe leaf symptoms (curl); plant considerably dwarfed
	4, 8	Latent A + mild yellow edge + crinkle	Severe leaf symptoms; plant greatly dwarfed
	5	Latent A + mottle + veinbanding	Severe leaf symptoms (curl); plant considerably dwarfed
	6	Latent A + mild yellow edge	Moderate leaf symptoms (leaves yellow); moderately dwarfed
	7	Latent A + latent C + mild yellow edge + veinbanding	Severe leaf symptoms (curl); plant considerably dwarfed

[a]Excised leaf-petiole graft technique[4] used to make inoculations.
[b]120 days after grafts made.
[c]Virus and/or virus complex "recovered" from inoculated plants by use of indicators.

RESULTS

The results of studies carried on are given in Table 1. As shown, all clones of F. chiloensis tested were uninjured by the principal Northwest strawberry viruses (Figure 1). However, in all cases the virus or virus complex introduced was "recovered" from inoculated plants by grafting excised leaves therefrom into susceptible indicator plants showing that, though highly tolerant, the species is readily susceptible to virus infection.

While minor differences were noted in the appearance (leaf characteristics) of the various clones of F. chiloensis collected from different locations, these differences are apparently unrelated to virus tolerance.

The same viruses caused visual leaf symptoms and a decrease in the size of the leaves of inoculated Marshall plants (Figure 2; and Table 1).

Such results imply that F. chiloensis contains genes that give the plant a useful degree of tolerance and may be a good source of virus tolerance in a strawberry breeding program.

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EFFECT OF FUNGICIDES AND INSECTICIDES ON THE GERMINATION OF CORN AFTER STORAGE[1]

C. O. Grogan[2], M. S. Zuber[2], H. E. Brown[3], M. D. Whitehead[4], and V. M. Stanway[4]

Summary

Cold- and standard-germination tests were made at 0, 3, 6, 12, and 18 months after treatment with fungicides, insecticides, and combinations to corn seed. Germinations obtained immediately after treatment showed little or no association with results from subsequent tests; whereas germination percentages obtained 3 months after treatment were generally highly correlated with germination percentages at later dates.

Fungicides applied by the slurry method did not lower germination appreciably until 6 months after treatment. Germination improved within 18 months.

Insecticides reduced germination rather rapidly until 6 months after treatment. There was generally little sign of recovery, indicating a rather permanent phytotoxic effect.

The germination of corn treated with insecticide-fungicide combinations was higher than of corn treated only with insecticides. The fungicides probably provided protection against fungi to seedlings weakened by insecticides.

The germination of corn treated with fungicide-insecticide combinations improved through the use of an adherent.

Aldrin appeared to have some fungicidal properties.

The treatment of seed corn with fungicides to control soil- and seed-borne diseases is a universal practice among seed-corn processors. Additional treatment with an insecticide to control soil insects is practiced by some members of the seed-corn industry. Certain insecticides may provide protection against storage insects as well as soil insects at planting time. It is well known, however, that insecticides are phytotoxic in varying degrees.

The phytotoxicity of a number of insecticides has been found (3, 4) to be reduced by including a fungicide in the seed treatment. It is the general opinion that the insecticidal treatment weakens the seed and predisposes it to fungal infection. The addition of a fungicide provides protection against fungus invasion and thus improves germination. It was reported (1) also that certain insecticides had no adverse effects on the fungicidal activity of some fungicides; and, conversely, that captan, dichlone, and thiram did not affect the activity of the insecticide dieldrin.

However, so far as the authors know, there is no published information on the effect of fungicides, insecticides, or fungicide-insecticide combinations on the germination of corn treated and stored for 3 or more months prior to planting. Unpublished results at the Missouri Agricultural Experiment Station (2, 5) indicate a differential response to treatments evaluated by the cold-test method.

The objective of the study reported was to determine whether such treatments applied in fall or early winter would adversely affect germination by planting time, usually several months later. Thus, the purpose of the study was not to compare fungicides, insecticides, and combinations individually, but their effect as a whole on the germination of stored corn.

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MATERIALS AND METHODS

Three fungicides, Arasan, captan, and Mathieson experimental 1562, and four insecticides, aldrin, dieldrin, heptachlor, and lindane, were applied individually and in combinations by the slurry method to untreated F_1 seed of Kansas 1639, of which WF9 X 38-11 is the female parent. In addition, an adherent was used in treatments involving captan. It was not possible to make all combinations of treatments with the adherent, as the materials and facilities were limited. The amounts applied were those recommended by the respective manufacturers (Table 1). The rate for insecticides was sufficient to protect corn from wireworm damage.

Table 1. Amounts of fungicides, insecticides, and adherent applied in the corn-seed-treatment study.

Material	Actual required	Formulation required
Adherent (benzol acetyl sucrose)	29.6 ml per bu	0.77 ml per 1.5 lb
75% Arasan	1.0 oz per bu	1.33 oz per bu 0.99 gm per 1.5 lb
50% captan	1.5 oz per bu	3.00 oz per bu 2.23 gm per 1.5 lb
50% 1562	1.3 oz per bu	2.60 oz per bu 1.95 gm per 1.5 lb
75% aldrin	2.0 oz per bu	2.66 oz per bu 1.98 gm per 1.5 lb
50% dieldrin	2.0 oz per bu	4.00 oz per bu 3.01 gm per 1.5 lb
50% heptachlor	1.3 oz per bu	2.60 oz per bu 1.95 gm per 1.5 lb
75% heptachlor	2.0 oz per bu	2.66 oz per bu 1.98 gm per 1.5 lb
25% lindane	1.0 oz per bu	4.00 oz per bu 3.01 gm per 1.5 lb

Corn-seed lots of 1 1/2 pounds each were placed in 1/2-gallon Mason jars with screw-type lids. The material and sufficient distilled water to make a slurry were added to each jar, and individual jars were then placed for 5 minutes in a machine which rotated them so that the material was distributed equally and evenly over each kernel. The seeds were removed from the jars immediately, dried overnight at room temperature, and returned to the respective jars for storage.

Germination percentages were determined by the standard- and cold-test techniques immediately after treatment and after 3, 6, 12, and 18 months of storage on the basis of four replications of 50 seeds each. The germination percentages reported are those for the germinated seeds classified as normal.

In the standard test, seeds from each treatment were placed between double thicknesses of moist paper toweling and rolled into a loose roll. The rolls were placed on end in a moist incubator and incubated at 30°C for 8 hours (day time) and 20° for 16 hours (night time) throughout the 7-day test period.

The cold test was conducted similarly except that a 1/8-inch layer of soil was sprinkled over the seed and incubated at 11°C for 5 days before being transferred to the standard-test incubator for an additional 5 days, after which the germinated seeds were counted.

The mean of the cold and standard tests generally was used in the comparisons. The mean of these two methods of testing would be more nearly comparable with actual field results than either alone.

EXPERIMENTAL RESULTS

The analyses of variance were computed on the number of seeds germinated, and the least significant differences were converted to a percentage basis. Highly significant differences were obtained for the different treatments, methods, dates, and the interactions. Table 2

Table 2. Summary of percent germination determined by the cold and standard methods of testing at 0, 3, 6, 12, and 18 months after treatment of F_1 seed of Kansas 1639 corn with all fungicides, insecticides, and combinations.

Material	Cold						Standard						Grand
	0	3	6	12	18	Mean	0	3	6	12	18	Mean	Mean
control	59.0	64.0	51.5	62.0	63.0	59.9	94.0	98.0	94.5	97.0	97.5	96.2	78.1
adherent	71.0	60.5	45.5	54.5	58.0	57.9	96.0	91.5	92.0	92.5	93.0	93.0	75.5
Arasan	71.5	58.0	53.5	35.5	62.0	56.1	95.0	96.0	93.5	92.5	94.5	94.3	75.2
captan	70.5	49.0	43.5	21.0	55.0	47.8	94.0	97.0	93.5	89.0	92.0	93.1	70.5
captan + adherent	66.0	77.0	65.5	55.5	74.5	67.7	95.0	97.0	95.0	94.0	95.5	95.3	81.5
1562	57.5	58.5	65.5	37.5	61.0	56.0	92.5	98.5	93.0	93.0	92.0	93.8	74.9
aldrin	68.5	50.0	37.0	28.0	33.5	43.4	94.0	90.0	96.0	92.5	90.0	92.5	68.0
aldrin + Arasan	73.0	74.0	62.5	67.5	81.5	71.7	99.5	97.0	94.5	95.0	95.0	96.2	84.0
aldrin + captan	67.0	64.0	54.5	38.0	78.5	60.4	95.0	98.0	95.0	94.5	95.0	95.5	78.0
aldrin + captan + adherent	63.0	74.5	58.5	47.5	79.0	64.5	94.0	94.5	92.0	94.5	93.5	93.7	79.1
aldrin + 1562	59.5	73.0	56.0	46.5	65.5	60.1	96.0	97.0	93.0	96.0	92.5	94.9	77.5
dieldrin	64.0	35.5	17.0	32.0	41.0	37.9	94.0	92.5	84.5	84.5	64.5	84.0	61.0
dieldrin + Arasan	59.0	70.5	44.0	56.5	75.5	61.1	93.0	93.5	88.5	93.0	89.5	91.5	76.3
dieldrin + captan	49.0	66.0	28.5	66.0	43.5	50.6	96.0	94.5	88.0	93.0	85.0	91.3	71.0
dieldrin + captan + adherent	67.0	74.5	45.5	68.5	65.0	64.1	95.0	97.0	90.5	91.5	95.0	93.8	79.0
dieldrin + 1562	55.5	62.0	48.0	56.5	71.0	58.6	94.0	95.0	90.5	95.5	89.0	92.8	75.7
heptachlor[a]	75.0	54.0	42.0	37.5	52.0	52.1	98.0	95.5	93.0	94.5	92.0	94.6	73.4
heptachlor + Arasan	70.0	66.0	61.0	52.5	58.5	61.6	88.0	91.5	93.5	92.0	90.0	91.0	76.3
heptachlor + captan	71.0	71.0	54.0	46.0	58.0	60.0	93.0	97.5	92.0	93.0	90.5	93.2	76.6
heptachlor + captan + adherent	67.0	67.5	62.5	56.0	61.0	62.8	97.0	96.5	91.0	96.5	93.0	94.8	78.8
heptachlor + 1562	56.0	66.0	49.5	68.5	63.5	60.7	96.0	96.0	92.5	95.5	92.5	94.5	77.6
heptachlor[b]	59.0	47.0	30.5	54.0	39.0	45.9	95.0	95.5	90.0	92.5	86.5	91.9	68.9
lindane	62.0	20.5	9.0	13.0	24.0	25.7	92.0	44.0	69.0	69.5	62.5	67.4	46.6
lindane + Arasan	65.0	39.0	34.0	38.0	62.0	47.6	87.0	48.5	81.5	68.5	79.0	72.9	60.3
lindane + captan	70.0	57.5	36.0	25.5	59.5	49.7	94.0	48.5	72.5	66.0	69.5	70.1	59.9
lindane + captan + adherent	60.5	50.5	39.0	46.5	65.0	52.3	93.0	95.0	89.5	85.0	74.0	87.3	69.8
lindane + 1562	62.0	40.5	32.0	32.0	61.0	45.5	88.0	63.0	80.5	76.0	80.0	77.5	61.5
L.S.D. at 5% level	20.7	20.7	20.7	20.7	20.7	6.9	20.7	20.7	20.7	20.7	20.7	6.9	6.9
Mean	64.4	58.9	45.4	46.0	59.7	54.9	94.0	88.8	89.6	89.5	87.5	89.9	72.4

L.S.D. at 5% level for methods of testing, 1.5; dates of testing, 3.1; methods of testing × dates of testing, 3.8; methods of testing × materials, 9.2; and for dates of testing × materials, 14.5.

[a]75% formulation.

[b]50% formulation.

gives the percent germination for the five dates of testing for the two methods and 27 treatments. The highest mean germination was immediately after treatment and declined at 3, 6, and 12 months, with a recovery appearing at 18 months. The range of the means for the different treatments by the cold-test method was 25.7 to 71.7, and the range for those of the standard test was 67.4 to 96.2. Among the treatments evaluated by the cold test, only aldrin plus Arasan was significantly higher than the control, but many others were arithmetically higher. Several treatments were significantly poorer than the control in the cold and standard tests, but the majority of these included lindane. There generally was little variation between dates for the control, and germination was as good at the end of the experiment as at the beginning. This would indicate that age of the seed did not influence the results. It would indicate also that corn seed can be expected to perform as well the second planting season as the first provided it is stored under good conditions.

All possible correlation coefficients were computed to obtain information on the association of germination percentages of the two methods of testing at each of the five dates. The correlation coefficients between corresponding dates of testing for the two methods were found to be significant at the 1 percent level except for the first date. This would indicate that satisfactory results might be obtained with either method after the first date, although the range within the dates of testing was greater for the cold test. The data suggest further that a germination test by either method 3 months after treatment would give a satisfactory indication of the results that might be obtained at a future date, with the possible exception of those obtained at 18 months by the cold-test technique. However, the most accurate information can be obtained by making actual germination tests for specific treatments at a given time.

Fungicides

The three fungicides used in this experiment gave similar results and did not lower germination appreciably until 6 months after treatment; but between 6 and 12 months, germination was reduced considerably. A marked recovery in germination occurred between 12 and 18 months.

Insecticides

Results from insecticidal treatments were much more variable than those from the fungicidal treatments. The most striking was the severe reduction of germination by lindane 3 months after the application of the material. The rate of decline of germination of all insecticidal treatments was considerable until 6 to 12 months after treatment. Recovery of germination at 18 months did not follow the consistent pattern found with the fungicides.

Fungicide-Insecticide Combinations

One of the main objectives of the study was to determine the effect of combinations of fungicides and insecticides on germination at different dates following treatment. The difference between insecticides in their reaction to combinations with fungicides was much greater than that of the fungicides. Differences between fungicides were slight, indicating any one of the three would be satisfactory for combining with the insecticides tested.

The germination patterns of seed treated with fungicides, insecticides, and combinations are compared in Figure 1. The combinations were 10 to 20 percent higher than the insecticides after the initial germination test. Therefore, the addition of a fungicide to an insecticide can be expected to overcome the reduction in germination encountered when treating seed with only insecticides. The germination of corn treated with such combinations will not differ appreciably from that of fungicidal treatments.

Adherent

Almost without exception the addition of the adherent was beneficial. The benefit from the addition of the adherent may explain the improvement in germination sometimes by re-treating seed 3 to 6 months after the original application. Figure 2 shows the average germination at different dates for all insecticidal treatments, all insecticides with captan, and all insecticides with captan and the adherent. The addition of an adherent improved germination at all dates after the first.

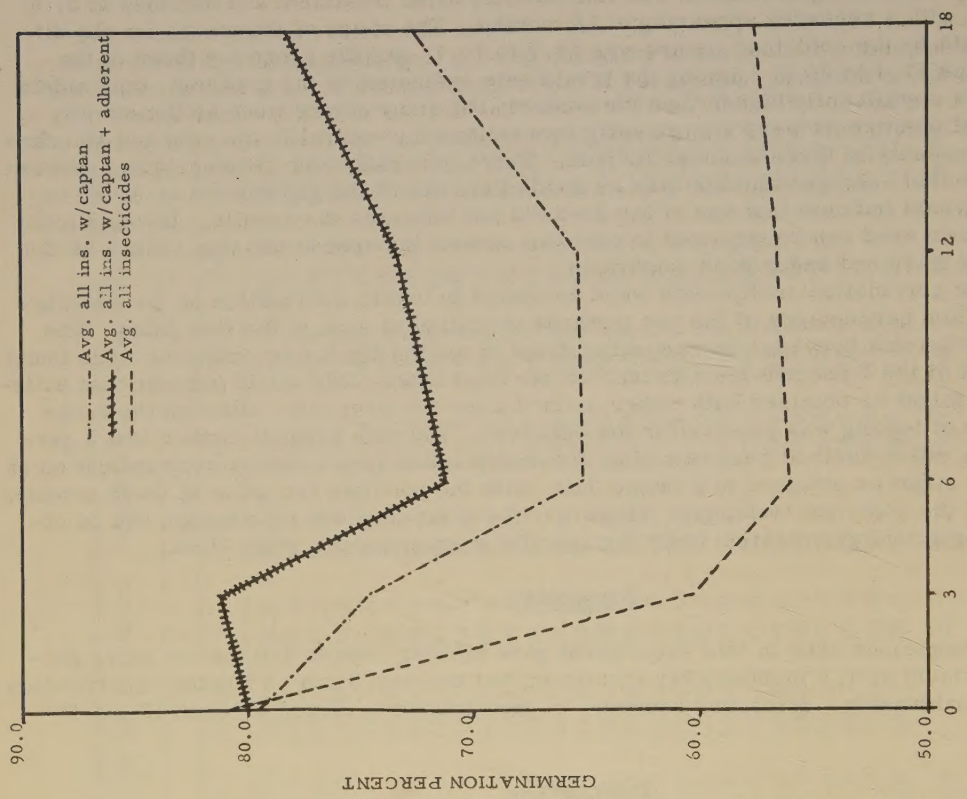

FIGURE 2. Average percent germination at 0, 3, 6, 12, and 18 months after treatment of F_1 seed of Kansas 1639 with captan in combination with all insecticides with and without an adherent, and the average of seed treated with all insecticides (combined cold and standard).

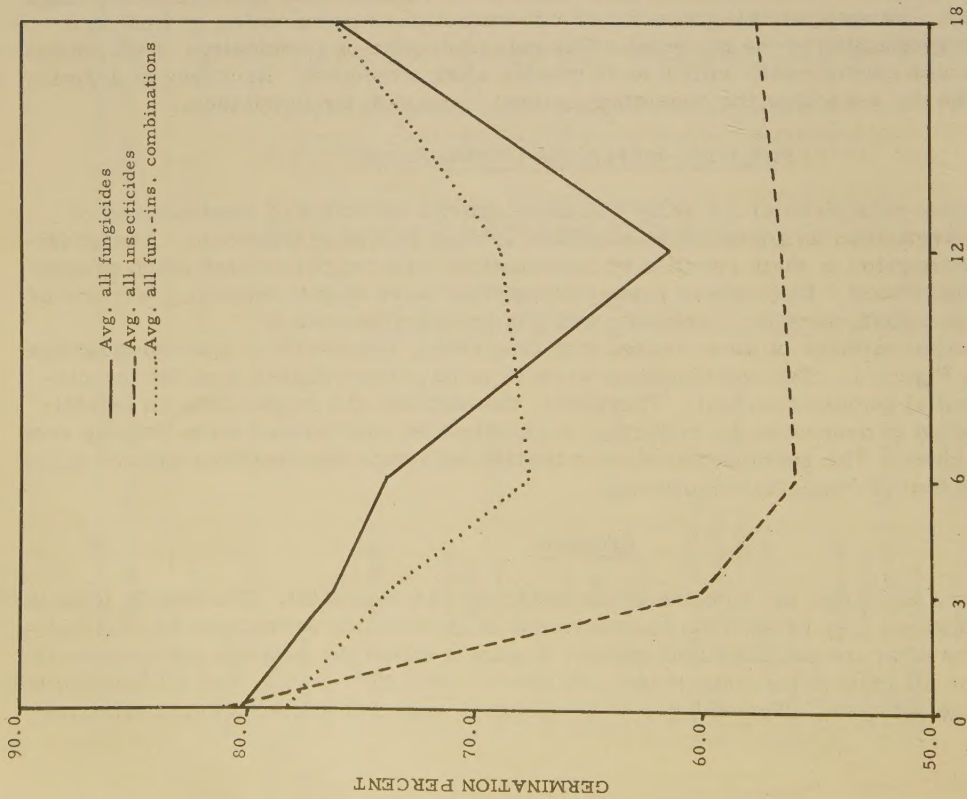

FIGURE 1. Average percent germination at 0, 3, 6, 12, and 18 months after treatment of F_1 seed of Kansas 1639 corn with all fungicides, insecticides, and fungicide-insecticide combinations (combined cold and standard).

DISCUSSION

Although the main objective of the study was the determination of the phytotoxicity of various insecticides and combinations of insecticides with fungicides applied directly to corn seed, a question which remains unanswered is how effective these various treatments would be in protecting corn against stored-grain insects. The answer to this question would involve infesting treated samples with various stored-grain insects, or the determination of the amount of insecticide residual remaining on the treated seed at various periods after treatment. However, neither was possible in the present study.

Another interesting point is whether any of the insecticides improved the effectiveness of certain fungicides. The cold-test germination increased significantly when aldrin was added to Arasan and captan before the seed was treated. However, when aldrin was considered individually, the cold-test germination was much lower than the control and many of the combinations. Thus, it appears that if aldrin has any fungicidal properties they are expressed only when aldrin is combined with certain fungicides. If the increase in germination resulted from some stimulating effect, it should be revealed in the standard test; but this was not apparent.

It is not clear why the corn treated with fungicides showed a recovery in germination at the 18-month testing date after a low germination at 12 months after treatment. The fact that the control and many of the insecticide treatments did not show a similar recovery rules out several possible explanations, including differing test conditions. Perhaps the phenomenon resulted from an inactivation of the fungicides which had previously induced a temporary partial dormancy. It should be pointed out that even though there was a temporary reduction in germination of fungicide-treated seed, this reduction was not significant until 6 months after the treatment of the seed. Therefore, corn planted in the spring after being treated with a fungicide in the fall should not be affected.

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NEW RECORD

A NEW HOST FOR VERTICILLIUM ALBO-ATRUM REINKE & BERTH.

By Craig R. Hibben[1]

Verticillium albo-atrum Reinke & Berth. (sensu Rudolph, Carpenter, and Wollenweber) has been successfully isolated from a 20- to 25-year-old *Acer pennsylvanicum* (Moosewood, Striped Maple) in Ithaca, New York. Typical wilting symptoms were noticed in 1957 and 1958, and the fungus was isolated in both these years. All branches with wilting leaves were removed in 1958. No wilting has been observed in 1959, but the fungus has again been isolated. All isolations have been from the smaller branches of the tree. No report of *Verticillium* infecting *Acer pennsylvanicum* has been found.

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